Neural correlates of cognitive impairment in patients with multiple sclerosis

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Summary

The estimated prevalence of cognitive impairment in multiple sclerosis (MS) ranges from 40 to 70%, affecting all disease stages and subtypes and having devastating effects on patients' everyday life. However, the pathological neuronal correlates are not clarified yet. Thus, this thesis addresses several open questions in the field of neural correlates of cognitive impairment in MS - with a core focus on cortical pathology - using structural magnetic resonance imaging (MRI) and an extensive, carefully selected set of neuropsychological tests. Three studies were conducted as part of the present thesis, based on a large cohort of 48 relapsing-remitting MS patients and 48 healthy controls.

Recent studies suggest that cortical lesions in MS are important contributors to clinical disease severity. Thus, study 1 was set out to compare MS patients with and without cortical lesions regarding cognitive functioning, but also regarding demographic and basic disease characteristics. The result showed that mnestic impairment is a typical feature of cortical affection in MS and propose a so-called 'cortically dominant' MS subtype.

Despite the increasing amount of correlational studies between neuroanatomical alterations and cognitive dysfunction in MS, topological studies on specific cognitive functions are very rare. Study 2 was set out to fill this gap by investigating neurostructural correlates of executive dysfunction in MS patients and healthy controls, both on a global and a regional level. The results reveal two important findings: First, on a behavioral level, we find that executive dysfunction in MS patients appears to specifically affect fluency. Second, we show a lateralized clinico-anatomical correlation pattern between fluency performance and cortical thickness in the anterior cingulate cortex, a finding previously described in brain-damaged patients with large lesions only, for example after stroke. The lateralized pattern described here appears novel and quite unique in the literature on MS, a disease affecting the two hemispheres in a comparable way and usually characterized by its widespread pathology.

Human subjects typically deviate systematically from randomness when attempting to produce a sequence of random numbers. Study 3 investigated, for the first time, the impact of MS on random number generation (RNG) and its neurostrucutral correlates. The behavioral results illustrate a loss of behavioral complexity in

the course of MS while imaging results argue for an association between cortical pathology - in terms of cortical lesions and atrophy - and RNG performance.
With these studies, we contribute to a better understanding of cognitive impairment in patients suffering from MS, highlighting the relevance of cortical pathology for cognitive functioning in MS and, thus, adding a small puzzle piece to this vast field of MS cognition research.

Zusammenfassung

Bei 40-70% der Patienten mit Multipler Sklerose (MS) treten im Verlauf der Erkrankung kognitive Defizite auf, unabhängig von Erkrankungsdauer oder -form. Diese Dysfunktionen können den Alltag und damit die Lebensqualität der Betroffenen erheblich beeinflussen. Dennoch sind die pathologisch-neuronalen Korrelate bis heute nicht gänzlich bekannt. Die vorliegende Doktorarbeit hat zum Ziel, mittels struktureller Magnet Resonanz Tomographie (MRT) und einer umfassenden Anzahl sorgfältig ausgewählter neuropsychologischer Tests offenen Fragen bezüglich neuronaler Korrelate kognitiver Beeinträchtigungen bei MS-Patienten nachzugehen. Der Schwerpunkt liegt dabei in der kortikalen Pathologie. Drei Studien wurden im Rahmen dieser Arbeit durchgeführt. All diese basieren auf einer grossen Stichprobe von 48 MS-Patienten und 48 gesunden Kontrollen.

Jüngste Studien haben gezeigt, dass kortikale Läsionen zur Schwere der Erkrankung beitragen. Daher wurden in der Studie 1 MS-Patienten mit kortikalen Läsionen und solche ohne miteinander verglichen; dies einerseits bezüglich kognitiver Funktionen, anderseits bezüglich demographischer Unterschiede oder anderer krankheitsbezogener Charakteristika. Es zeigte sich, dass mnestische Defizite kennzeichnend sind für eine kortikale Beteiligung der Krankheit. Daher schlagen wir einen "kortikal-dominanten" Subtyp der MS vor.

Trotz einer zunehmenden Anzahl Korrelationsstudien, die den Zusammenhang zwischen strukturellen Veränderungen und kognitiven Defiziten untersucht haben, fehlt es an topologischen Studien, die sich auf spezifische kognitive Defizite beziehen. Mit Studie 2 wurde versucht diese Lücke zu schliessen. Es wurden neurostrukturelle Korrelate der Exekutivfunktionen bei MS-Patienten und gesunden Kontrollpersonen untersucht, und zwar sowohl auf globaler als auch auf fokaler Ebene. Zwei wichtige Befunde ergaben sich: Erstens zeigte sich auf der Verhaltensebene, dass exekutive Dysfunktionen bei MS-Patienten ausschliesslich die Ideenproduktion betreffen. Zweitens, fanden wir ein lateralisiertes klinisch-anatomisches Korrelationsmuster zwischen Leistung in der Ideenproduktion und kortikaler Dicke im anterioren cingulären Kortex. Ähnliche Ergebnisse wurden bereits bei hirngeschädigten Patienten mit grossen fokalen Läsionen gezeigt (z.B. Schlaganfallpatienten). Das beschriebene lateralisierte Muster ist hingegen neu und einzigartig in der Literatur betreffend MS, eine Krankheit, die beide Hirnhemisphären in vergleichbarer

Weise schädigt und charakterisiert wird durch ihre ausgedehnte Pathologie.
Beim Versuch zufällige Zahlensequenzen zu produzieren weichen Menschen typischerweise systematisch von der theoretischen Zufälligkeit ab. Studie 3 untersuchte, zum ersten Mal, den Einfluss von MS auf die Zufallsgenerierung von Zahlen und deren neurostrukturelle Korrelate. Die Resultate ergaben einen Verlust an Verhaltenskomplexität bei MS Patienten. Die bildgegebenden Analysen zeigten einen Zusammenhang zwischen kortikaler Pathologie (im Sinne von kortikalen Läsionen und Atrophie) und der Leistung in der Zufallsgenerierung von Zahlen.
Mit diesen drei Studien verbessern wir das Verständnis kognitiver Beeinträchtigungen bei MS-Patienten, indem die Bedeutung kortikaler Pathologie für die geistige Leistungsfähigkeit herausgestrichen wird. Hiermit leisten wir einen kleinen Beitrag im umfassenden Gebiet der MS Kognitionsforschung.

Abbreviations

BICAMS	Brief International Cognitive Assessment for Multiple Sclerosis
BPF	Brain parenchymal fraction
CIS	Clinically isolated syndrom
CL	Cortical lesion
CNS	Central nervous system
CSF	Cerebrospinal fluid
DIR	Double inversion recovery
DMD	Disease modifying drug
EBV	Epstein-Barr virus
EDSS	Expended Disability Status Scale
FDR	False discovery rate
FLAIR	fluid attenuated inversion recovery
FOD	First-order difference
GM	Grey matter
HC	Healthy control
MDT	Mental Dice Task
MPRAGE	Magnetization-prepared rapid gradient-echo
MRI	Magnetic resonance imaging
MS	Multiple Sclerosis
nCL	non-cortical lesion
NPM	Non-parametric mapping
PASAT	Paced Auditory Serial Addition Test
PPMS	Primary progressive multiple sclerosis
RNG	Random number generation
RRMS	Relapsing-remitting multiple sclerosis
ROI	Region of interest
SBM	surface-based morphometry
SPM	Statistical Parametric Mapping
SPMS	Secondary progressive multiple sclerosis
TPI	Turning point index
TVW	Third ventricle width
VLSM	Voxel-based lesion symptom mapping
WEIMuS	Würzburg fatigue inventory
WM	White matter

Contents

1 Introduction

"A cette expression dominante de la physionomie correspond presque toujours un état mental qui mérite d'être signalé. Il y a un affaiblissement marqué de la mémoire; les conceptions son lentes; les facultés intellectuelles et affectives émoussées dans leur ensemble."

Jean Martin Charcot, 1877

Since this first description of cognitive impairment in patients suffering from multiple sclerosis (MS) by Jean-Martin Charcot - the founder of modern neurology - a lot of work has been done. In the second half of the 20th century, it became more and more evident that cognitive impairment is a frequent feature of MS and clinicians have become increasingly aware of the profound functional impact of cognitive impairment in MS. During the past three decades, much effort has been put into the characterization of the extent, type and reasons of cognitive disability in patients with MS. The main driving forces of research in this area is the need to better explain the heterogeneous and complex manifestation of cognitive impairment in MS patients in order to develop potential prevention and novel therapeutic interventions. Through these studies, many advances have been made concerning our understanding of cognitive impairment in MS. However, many aspects still need to be considered.

This doctoral thesis tries to extend our knowledge about cognition and the neural underpinnings in patients suffering from MS. The focus will be on several aspects of structural magnetic resonance imaging to investigate neuroanatomical correlates of cognitive impairment in MS patients, with a core focus on cortical pathology. The following section (Chapter 2) addresses the question what MS is and outlines the theoretical background and previous research, which was derived in the field of cognition and structural imaging in MS. Chapter 3 outlines the aims of the empirical work and emphasizes the relevance of this thesis. Chapter 4 describes the study sample and gives a short introduction into the methods used in the three studies conducted as part of the present doctoral thesis. Chapter 5 includes the

the three empirical studies comprising their results and discussion. To conclude, Chapter 6 gives a summary of the results and a general discussion.

2 Theoretical background

2.1 Epidemiology, etiology and subtypes of MS

Multiple sclerosis means 'multiple scarring' or 'hardening' (Wiendl & Kieseier, 2010) and is also know as 'disseminated sclerosis' or 'encephalomyelitis disseminata' (Netter, 2007). It is one of the most common disabling neurologic disease in young adults, affecting more than 10'000 people in Switzerland (Multiple Sclerosis Society of Switzerland, 2015). Generally, MS has a prevalence ranging from 2 to 150 in 100'000 people (Rosati, 2001), depending on the country or specific population (Koutsouraki, Costa, & Baloyannis, 2010). In contrast to other neurological conditions, MS has an early onset in young adult life, with symptoms typically beginning in the third or fourth decade of life. The disease shows a female predominance of 2:1 to 3:1 (Chiaravalloti & DeLuca, 2008).

The etiology of MS is thought to be multifactorial. Epidemiological data indicate that both environmental (e.g. latitude, vitamin D, smoking, Epstein-Barr virus) and genetic factors are important contributors to disease development (Ramagopalan, Dobson, Meier, & Giovannoni, 2010), however, the specific elements remain unclear. Genetic susceptibility explains the clustering of MS cases within families, whereas the changes in MS risk that occur with migration can be explained only by changes in the environment (Ascherio, 2013). A recent metaanalysis about the environmental risk factors identified a biomarker of Epstein-Barr virus (anti-EBNA IgG seropositivity), infectious mononucleosis, and smoking to show the strongest consistent evidence of an association with disease development (Belbasis, Bellou, Evangelou, Ioannidis, & Tzoulaki, 2015). In contrast, a protective effect on the risk of MS has been proposed for vitamin D (Munger et al., 2004; Salzer et al., 2012).

Three main clinical subtypes of MS have been identified (Figure 2.1). Relapsing-remitting MS (RRMS), affecting about 85% of the newly diagnosed patients, is characterized by clearly defined attacks - often called relapses - of worsening of symptoms. Between the relapses, substantial or complete recovery are noted. Over time, the majority of RRMS patients (80%) enter a phase in which there is continuous deterioration, known as secondary-progressive MS (SPMS), i.e. a gradual decline with or without occasional relapses or remissions. Approximately

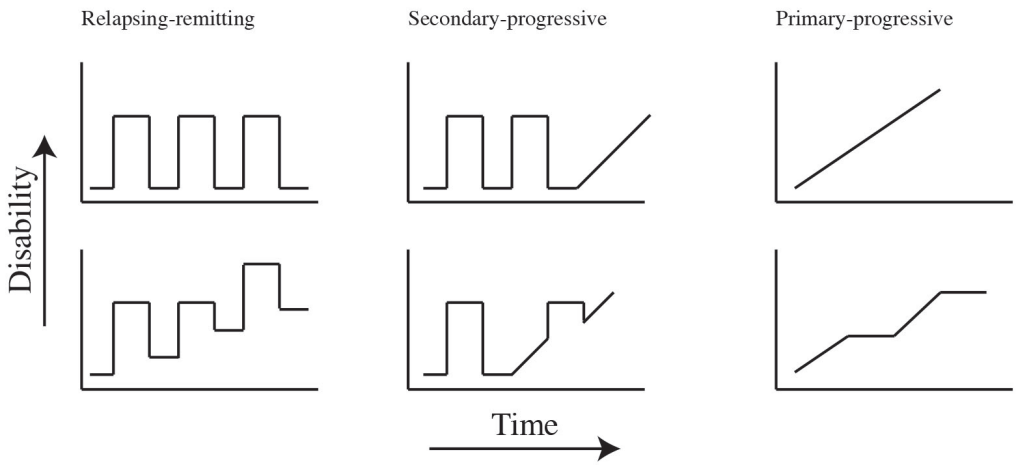

Figure 2.1: Graphs of the three main MS subtypes.

10 to 15% of patients experience a primary progressive (PPMS), relatively non-inflammatory disease course from the onset with a characteristic steady worsening of neurologic functioning, without any distinct relapses or periods of remission. Furthermore, clinically isolated syndrome (CIS) is a term describing a first clinical episode in which patients have symptoms and signs suggestive of an inflammatory demyelinating disorder of the central nervous system (CNS) (Miller, Chard, & Ciccarelli, 2012). Often, but not always, it progresses to MS.

2.2 Pathophysiology

After more than a century, of research the precise pathogenesis of MS still remains unclear. The full complexity of the ongoing debate of the pathophysiology of MS is beyond the scope of this thesis, but the most important findings are shortly introduced in order to provide a background for the subsequent sections.
Traditionally, MS is considered to be an autoimmune inflammatory disorder (E. Fox, 2004), in which dysregulated auto-reactive T cells in the periphery cross into the CNS through a pathological disruption of the blood-brain barrier and, together with macrophages and B cells, proceed to destroy CNS elements, particularly myelin (Stys, Zamponi, van Minnen, & Geurts, 2012). In areas of demyelination, sclerotic plaques are formed and the normal transmission of the action potential might be disrupted. This disseminated focal demyelinating lesions in the white matter (WM) are the pathological hallmark of MS. However, by now it is known, that MS have effects beyond the loss of the myelin sheath (Purves et al., 2008). Inflammatory demyelination can lead to irreversible axonal and dendritic damage, transection and loss, defined as secondary degeneration. Beyond that, it has been proposed lately that MS might be even a degenerative disorder such as Alzheimer's

and Parkinson's disease (Hauser & Oksenberg, 2006; Trapp & Nave, 2008), where primarily the axon selectively degenerate, followed by demyelination in the secondary phase. Evidence for this primary degenerative process comes from careful pathological examinations showing that outer myelin wraps are often still intact, whereas myelin abnormalities are observed at the inner myelin sheath. Similar to the indisputable inflammatory character of the disease which has been challenged, the traditionally view of MS as a WM disease has been faced. Thus, in the recent years, it became clear that the grey matter (GM) is not spared. However, the underlying mechanisms inducing GM pathology remains unclear. Since the first evidence of cortical involvement in MS, there is an ongoing debate about the extent to which GM pathophysiology is similar to that in WM. Recently, several mechanisms including either primary (arising within GM regions) or secondary (as a result of ongoing damage in the WM) pathogenic processes have been proposed. One of the main theories about the pathogenesis of GM lesions suggests that meningeal inflammation and associated myelinotoxic agents that might be produced in the meninges and then diffuse into the cortex could be causally related to cortical demyelination (Geurts, Calabrese, Fisher, & Rudick, 2012). Other alternative theories proposed several secondary disease mechanisms, where GM damage results from ongoing damage in the WM. Calabrese and colleagues (2015) conclude in their very recent review about the origins of GM damage in multiple sclerosis, that GM damage proceeds in a manner that is partly independent of WM damage, although they assume clearly some degree of relationship between the two types of damage. Support for an independent mechanisms is coming from MS cases exhibiting predominantly GM pathology with little WM involvement, and vice versa. Thus, it seems that the inflammatory processes affecting the GM occurs, at least partly, independent of WM inflammation. Furthermore, they report inflammatory and neurodegenerative mechanisms involved in GM pathology. However, the relationship between the two remains unclear. It can be concluded that the pathophysiology of MS seems to be a complicated picture that remains to be elucidated.

2.3 Structural imaging and neuroanatomical changes in MS

Although computed tomography (CT) can in some cases depict hypodensitiy within particularly severe MS lesions, magnetic resonance imaging (MRI) is considered the most sensitive imaging modality for MS (R. Fox, 2008). Different tissue types (e.g. GM, WM, cerebrospinal fluid (CSF)) and tissue alterations ex-

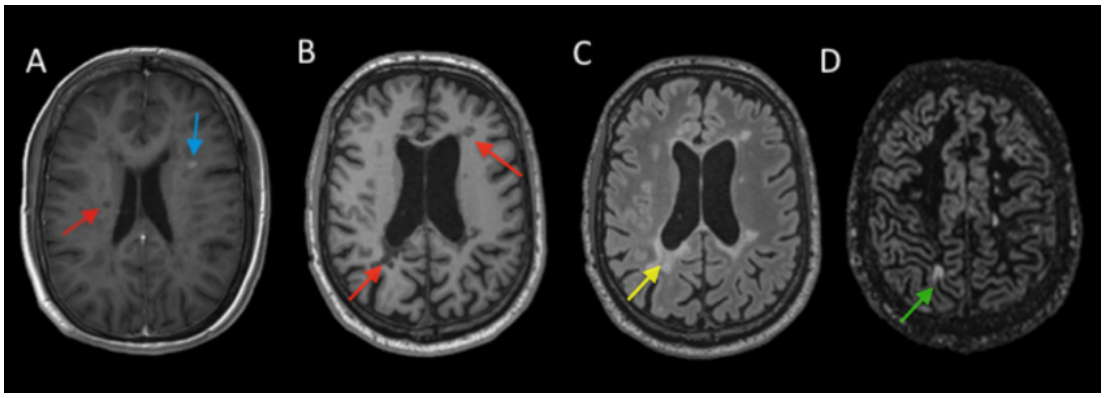

Figure 2.2: Different MRI scans from MS patients showing characteristic lesions. A) T1-weighted image showing a *black hole* (red arrow) and hyperintense gadolinium-enhanced lesion (blue arrow) B) Another T1-weighted image from a MPRAGE (Magnetization prepared rapid gradient echo) scan with *black holes* (red arrows). C) T2-weighted image from a MS patient, showing characteristic lesions, so called T2-lesions or plaques (yellow arrow) D) DIR sequence showing cortical lesions (green arrow). Source: Geisseler (2012)

hibit differential magnetic properties resulting in this high sensitivity for structural changes in MS. Its ability to depict the pathological features in exquisite detail, conventional MRI is widely applied to ascertain the diagnosis in patients suspected of MS and plays an important role in monitoring the evolving disease in vivo. Furthermore, it has become a major outcome measure in clinical trials and other scientific investigations (Barkhof, 2002). In fact, it is incorporated into the formal diagnostic algorithm for MS since 2001 (McDonald et al., 2001) - with new lesions fulfilling either dissemination in space or dissemination in time criteria (Bakshi et al., 2008) - and has an established role in the exclusion of clinically similar conditions (Charil et al., 2006). In the following two sections, the most important neuroanatomical changes in MS patients assessed with MRI will be introduced.

2.3.1 Focal lesions

Conventional MRI in the clinical routine includes T1- and T2-weighted sequences, the lalter with and without gadolinium-based contrast agent (Figure 2.2). Inflammation and demyelination within the CNS causes a prolongation in the T2-relaxation time, resulting in focal areas of hyperintensity on T2-weighted images (Figure 2.2 C) (Barkhof & van Walderveen, 1999). These signal changes are so called T2-lesions or plaques - the hallmark of MS. When the tissue injury is severe, MS lesions can cause T1-prolongation, which appears dark on T1-weighted images (R. Fox, 2008). These 'black holes' represent irreversible tissue destruction and axon loss (Bermel & Bakshi, 2006) (Figure 2.2 A and B). Gadolinium-enhanced T1-weighted MR images allow to distinguish active inflammatory lesions - indicative for a relapse - from inactive lesions, since enhancement occurs as a result of

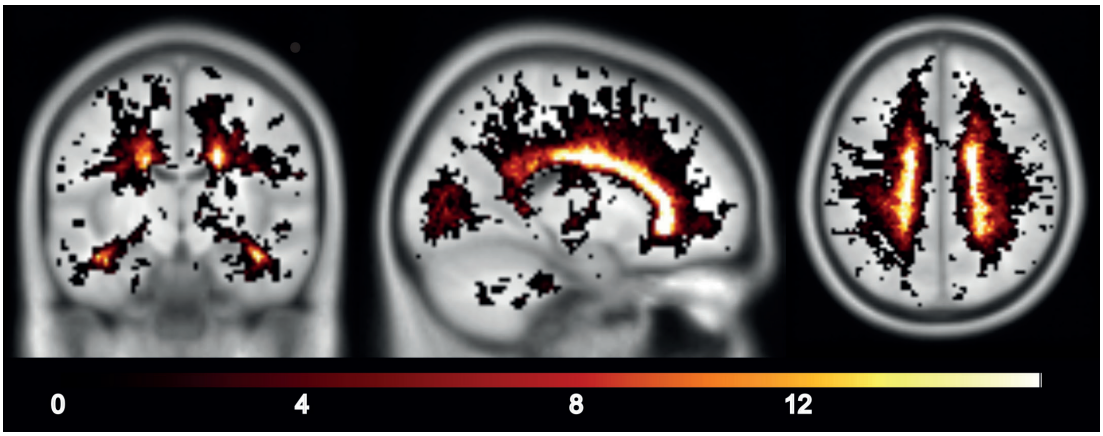

Figure 2.3: Spatial distribution of T2-hyperintense lesions in 48 MS patients based on normalized lesion. Lesion frequency across the sample is displayed for every depicted voxel. Color bar indicates the number of patients showing damage to a particular voxel. Source: Unpublished data from University Hospital Zurich

increased blood-brain barrier permeability (Filippi & Rocca, 2011) (Figure 2.2 A, blue arrow). Although lesions can occur anywhere in the CNS, there are certain sites of predilection (McDonald & Ron, 1999). WM lesions have been predominantly found in near the borders of the lateral ventricles (periventricular), as illustrated in Figure 2.3. The classical view of MS as a pure WM pathology has not been overcome until the beginning of the 21^{st} century, even though first histological reports of demyelinated foci in the cortex of patients with MS date back to the beginning of the 20^{th} century (Dawson, 1916). By now, grey matter involvement in the pathology of MS is well established (for reviews see Geurts & Barkhof, 2008; M. Calabrese, Magliozzi, et al., 2015) and is recognized as s feature in most MS patients. The traditional view of MS as a WM disease was driven by the higher sensitivity of the conventional MRI techniques to WM changes (Horakova, Kalincik, Blahova Dusankova, & Dolezal, 2012). Cortical lesions are typically relatively small, have longer relaxation times than those of WM (Kidd et al., 1999) and have poor contrast against the surrounding normal GM (Bagnato et al., 2006). A significant improvement in the in vivo detection of GM lesion was obtained by a novel MRI technique, called double inversion recovery (DIR) sequences (Figure 2.2). It shows an improved sensitivity to GM changes by suppressing the signal from the CSF and the WM (Filippi & Rocca, 2011). Even though, a recent study has shown that DIR sequences detect only a minority of cortical lesions in MS (Seewann et al., 2012), it is currently considered to be the best pulse sequence for cortical lesion detection and still has an excellent pathologic sensitivity.

In recent years, much effort has been put into describing and classifying pathology within GM areas, resulting in a currently widely used scoring system that distinguishes between four major cortical lesion types. Type I lesions are combined

WM/GM lesions. Type II lesions are entirely located within the cerebral cortex and are not in direct contact with subcortical WM. Type III are subpial cortical demyelination, reaching from the pia downwards into the cortex. Lesions comprising the entire width of the cortex, without spreading in the subcortical WM are categorized as type IV lesions (Bø, Vedeler, Nyland, Trapp, & Mørk, 2003a, 2003b). Cortical lesions have been reported to vary significantly among the different cortical areas, being the cingulate gyrus, the temporal and frontal cortices the mostly affected (Bø et al., 2003b, e.g). Although cortical lesions can be seen at all stages of the disease their incidence and size increases with disease duration and accumulate over time (M. Calabrese, Rocca, et al., 2010).

2.3.2 Atrophy

In addition to focal lesions, another well-known feature of MS is brain atrophy (Barkhof, 2002), affecting not only the WM, but also the GM. Until the beginning of the 21^{th} century, it was difficult to quantify changes in brain volume with any precision. Improvements in methods to assess brain volumes from conventional MRI scans have allowed to sensitively and reproducibly quantify the extent of tissue loss in GM and WM separately and to study the distribution of atrophy at a regional level.

Most easily noticeable is the presence of diffuse global atrophy as reflected by enlargement of the ventricular system and sulcal widening (Sicotte, 2011) (Figure 2.4 B). However, a reduction of global white and grey matter volume has been observed, too. Furthermore, it has been established that corpus callosum atrophy occurs in MS and that it can be reliably measured (Figure 2.4 A). Whereas the detection of focal GM lesions was, and still is, challenging using conventional MRI sequences, GM atrophy can be robustly and reliably measured form standard MRI (Fisher, Lee, Nakamura, & Rudick, 2008) using various methods. The involvement of both, cortical and several subcortical GM structures, such as the thalamus (Houtchens et al., 2007) or hippocampus (Sicotte et al., 2008), has been demonstrated. For the first time, Sailer and colleagues (2003) described changes in absolute thickness of the cortical ribbon of MS patients. On the one hand, they showed a significant overall thinning of the cerebral cortex in MS patients compared to controls, on the other hand, they described a distinct distribution of focal atrophy. Supporting these early findings, several more recent studies showed a in-homogeneously distribution of cortical atrophy (Benedict et al., 2005; M. Calabrese, Rinaldi, et al., 2010; Riccitelli et al., 2011; Tekok-Kilic et al., 2007) with a predominant involvement of temporal and frontal regions.

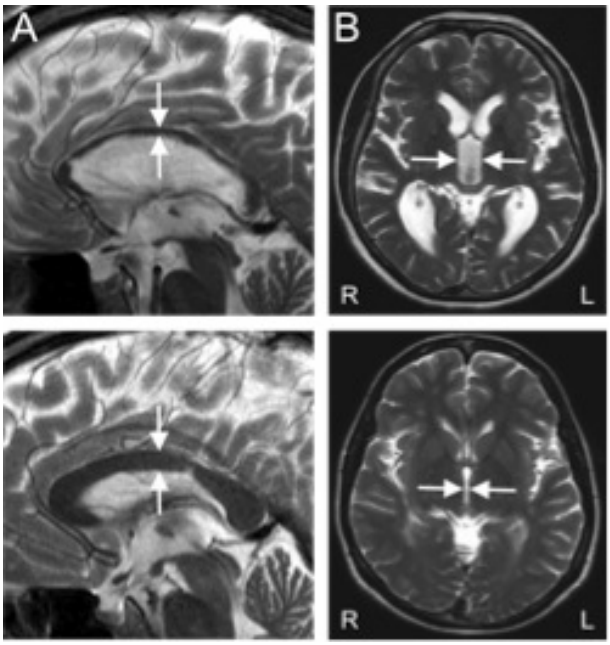

Figure 2.4: MRI markers of MS-related brain atrophy. Based on two RRMS patients, one with considerable atrophy (upper row), the other with no atrophy (lower row). A) Mid-sagittal T2-weighted images depicting prominent thinning of the corpus callosum (white arrows) in the upper patient. B) Axial T2-weighted images illustrating ventricular widening including the third ventricle (white arrows) in the upper patient. Moreover, sulcal widening is visible in the region of the left Sylvian fissure. Horizontal image orientation follows the radiological convention (right on left side). Source: Pflugshaupt et al. (2015)

2.4 Clinical presentation of MS

One of the characteristics of MS is the variability of its symptoms, as it affects all parts of the CNS. No two patients have exactly the same symptoms, and each person's symptoms can change or fluctuate over time, indicating also a high intra-individual variability. However, despite the broad range of symptoms, some of them are more prevalent than others. Common MS symptoms are walking (gait) disorders, weakness, vision disturbance, numbness or tingling, dizziness, vertigo, bladder or bowel dysfunction (Kesselring & Beer, 2005). In addition to the neurological symptoms that characterize MS, the disease is associated with a range of behavioral disorders. These include depression, fatigue, cognitive impairment, bipolar affective disorder, anxiety disorders, euphoria, pathological laughing and crying as well as psychosis (Feinstein, DeLuca, Baune, Filippi, & Lassman, 2013). The most common behavioral disorder is major depression, which is three times more prevalent in MS patients relative to the general population (Siegert & Abernethy, 2005). However, fatigue is the most common complaint of MS generally, independent of the clinical subtype and the stage of the disease (MacAllister & Krupp, 2005). According to different studies, 53 to 90% of patients with MS experience cognitive and/or physical fatigue (Krupp, LaRocca, Muir-Nash, & Stein-

berg, 1989; Krupp, Alvarez, LaRocca, & Scheinberg, 1988; Penner et al., 2007). Cognitive fatigue is defined as a decline in cognitive performance over a brief period of time, such as a neuropsychological testing session lasting approximately 2 hours or part of a workday (Julian, 2011), whereas physical fatigue is defined as a sense of exhaustion, lack of physical energy, or tiredness (Krupp, 2006). Fatigue is frequently reported by patients to be the most disabling symptom of the disease (Giovannoni, 2006), probably as it interferes with work, family and social activities (Rocca et al., 2014). In addition to the symptom variability, also the course of MS is highly variable and unpredictable. On the one hand, rare cases are fatal in less than a year, on the other hand, occasional patients have little disability even after 50 years of disease (McDonald & Ron, 1999). However, often there is a substantial correlation between physical disability and disease duration.
All these functional deficits, including the physical disability and neuropsychiatric symptoms, are determinants of quality of life (DeLuca & Nocentini, 2011; Mitchell, Benito-Leon, Gonzalez, & Rivera-Navarro, 2005; Penner, Kappos, Rausch, Opwis, & Radu, 2006) and lead to a loss of independence and restriction in social activities. Furthermore, they are strongly associated with work disability (Bøe Lunde et al., 2014; Strober et al., 2012) and cause in up to 80% of the cases unemployment within a ten-year disease course (Rao, Leo, Ellington, et al., 1991).

Krutzke (1983) developed the Expended Disability Status Scale (EDSS) for quantifying and evaluating disability resulting from MS. It is the most widely accepted and applied neurological rating scale for MS. The EDSS provides a score on a scale ranging from 0 (normal neurologic examination) to 10 (death due to MS) incrementing in 0.5 units. The scale measures impairment or activity limitation based on the examination of seven functioanl systems: Pyramidal, cerebellar, brainstem, sensory, bowel and bladder function, visual function, cerebral (or mental) functions. Between 0 and 4.5 people are able to walk without assistance, EDSS steps 5.0 to 9.5 are defined by the impairment to walk.

2.5 Cognition in MS

Already Jean-Martin Charcot described in his pioneer work cognitive impairment in patients with MS (Charcot, 1877). However, the topic generated little interest, and cognitive deficits have largely been neglected for many decades. This may be due to the observation that MS patients rarely show classical neuropsychological syndromes such as agnosia, aphasia, apraxia, or acalculia (Rao, 1986). Only lately, awareness of cognitive dysfunction in MS patients has increased and clinicians have become increasingly aware of the profound functional impact of

cognitive impairment in MS. During the last three decades, essential contributions to the understanding of cognitive deficits were made. By now, it is known that 45-70% of the MS patients are cognitively impaired (Amato, Zipoli, & Portaccio, 2006; Benedict, Cookfair, et al., 2006a; Rao, Leo, Bernardin, & Unverzagt, 1991). Cognitive deficits can appear in all disease stages and subtypes (Langdon, 2011). Beside RRMS, SPMS and PPMS, even in the earliest manifestations of the disease (CIS), cognitive deficits can be observed (Potagas et al., 2008). However, cognitive impairment is more prevalent and severe in progressive forms of MS compared to RRMS (DeLuca & Nocentini, 2011) and generally tend to increase over time (Amato et al., 2006), but more slowly and less consistently than in neurodegenerative disorders such as Alzheimer. Amato and colleagues (2001) showed in a longitudinal study that the prevalence of cognitive dysfunction increased from 26% to 56% in sample of MS patients over a period of 10 years. Remission of cognitive symptoms is uncommon.
Cognitive dysfunctions often result in significant functional impairment at work and at home, sometimes despite minimal physical disability (Elsass & Zeeberg, 1983). There are studies showing cognitive impairment in patients with little or no physical disability, usually assessed by the EDSS (Beatty, Goodkin, Hertsgaard, & Monson, 1990; Franklin, Nelson, Filley, & Heaton, 1989; Marsh, 1980). Moreover, there are observations that cognitive problems are frequently present even when other signs and symptoms of MS are absent in the neurologic examination. However, no clear associations have been found between cognitive dysfunction and measures of physical disability (Elsass & Zeeberg, 1983).

The extend of cognitive decline is wide. The question why some MS patients develop severe impairment in cognitive abilities, while cognitive functions remain intact in others is still unanswered (Benedict & Zivadinov, 2011). Factors such as age, gender, age at disease onset, level of education and cognitive reserve may have an influence on cognitive abilities and cause the extreme variability of cognitive impairment (Horakova et al., 2012). Advanced age and depression are discussed as potential risk factors for cognitive decline in MS patients, while consistent with the cognitive reserve hypothesis a high premorbid intellectual level of MS patients can protect from cognitive decline (Sumowski, Wylie, Gonnella, Chiaravalloti, & Deluca, 2010). The cognitive reserve hypothesis states that intellectual enrichment lessens the negative impact of neurological disease on cognitive status (Stern, 2009). Rare cases, in which cognitive impairment resembles dementia, are known (Julian, 2011), yet severe dementia in accordance with the criteria of the ICD-10 is relatively uncommon.
Another controversy concerns the relation of cognitive impairment to other fre-

quent MS symptoms, such as fatigue or depression. There are studies showing depression, anxiety and fatigue aggravate symptoms. Early studies have generally not found an association between depression and cognitive impairments in MS. Whereas a very recent study has found significant positive correlations between self-reported fatigue and cognitive test performance (Neumann et al., 2014), others failed to show such association (Morrow, Weinstock-Guttman, Munschauer, Hojnacki, & Benedict, 2009).

2.5.1 Impaired cognitive domains

The heterogeneity of the cerebral pathology of MS understandably results in great variety of cognitive problems (Benedict & Zivadinov, 2011; Huijbregts, Kalkers, de Sonneville, de Groot, & Polman, 2006). MS-related cognitive function affect various aspects of cognition. Probably the most consistently reported and primary cognitive deficit in MS is slowed information processing speed, which is well documented in many studies (e.g., Chiaravalloti & DeLuca, 2008; Roth, Denney, & Lynch, 2015; DeLuca, Chelune, Tulsky, Lengenfelder, & Chiaravalloti, 2004; Denney, Gallagher, & Lynch, 2011) and was already described by Charcot (1877). Patients have difficulty to think quickly or to keep up with conversation, and in neuropsychological assessments, they complete substantially fewer items on tests such as the Paced Auditory Serial Addition Test (PASAT; Kujala, Portin, Revonsuo, and Ruutiainen (1995)) or Symbol Digit Modalities Test (Parmenter, Shucard, & Shucard, 2007). Simplified, they feel "slowed down" mentally (Fischer, 2001, p. 236). Decrements in information processing speed are associated with other cognitive deficits common in MS, such as working memory and long-term memory (Chiaravalloti & DeLuca, 2008).
The other cognitive domain most commonly affected in patients with MS is anterograde episodic memory. Moreover, impaired memory is the most investigated cognitive deficit in MS and is regularly observed in MS patients - sometimes even reported as the most frequently disrupted cognitive domain (Rogers & Panegyres, 2007). There has been disagreement on the nature of MS-related memory impairment. In the 1980s, it has been suggested that cognitive impairment in MS becomes manifest as subcortical dementia (Beatty, Goodkin, Monson, & Beatty, 1989; Rao, 1986), which is characterized by slowness of mental processing, forgetfulness, impaired conceptual reasoning and planning, apathy, and depression, in the absence of agnosia, aphasia, dense amnesia, which are typical signs for cortical dementias such as Alzheimer's disease (Cummings & Benson, 1984). Thus, early work has suggested difficulty in retrieval as the core deficit in memory. However, the view of MS-related cognitive impairment as a variant of subcortical dementia was disputed

(P. Calabrese & Penner, 2007) by studies showing that memory problems are not restricted to difficulties in retrieving information (e.g. forgetfulness) but can also affect initial learning of information (DeLuca, Barbieri-Berger, & Johnson, 1994; Deluca, Leavitt, Chiaravalloti, & Wylie, 2013). Furthermore, problems in memory span (Thornton & Raz, 1997) or recognition (DeLuca, Gaudino, Diamond, Christodoulou, & Engel, 1998) have been observed. Memory impairment seems much more global than implied by the concept of subcortical dementia (Thornton & Raz, 1997). Moreover, and as elucidated above, neuroimaging studies highlighted cortical affection of the MS pathology, putting the usefulness of the term 'subcortical dementia' into question, when describing cognitive impairment in MS. Studies on executive functions - the cognitive abilities needed for complex goal-directed behaviour and adaptation to environmental changes or demands - in MS are rare, even though impairments in a broad range of executive functions have been observed (Arnett et al., 1994; Parmenter, Zivadinov, et al., 2007). Fifteen to 20% of patients suffer from impaired executive dysfunction (Drew, Tippett, Starkey, & Isler, 2008). Substantial deficits have been shown by Foong and colleagues (1997) in verbal fluency, interference, cognitive estimation, spatial working memory, generating strategies and planning. Furthermore, executive deficits were reported in semantic encoding and planning (Arnett et al., 1997) as well as in inhibition and set shifting (Drew et al., 2008). Henry and Beatty (2006) conclude that measures of fluency are amongst the most sensitive markers of cognitive impairment in MS.
However, not all cognitive domains are affected in MS. General intellectual functions (Macniven et al., 2008) and basic language skills such as word naming or verbal comprehension are often spared, even in the advanced stages of the disease (Rogers & Panegyres, 2007; Chiaravalloti & DeLuca, 2008). Also, simple attention skills (e.g. repeating digits) are typically unaffected.

2.5.2 Neuropsychological assessment in MS

A formal neuropsychological assessment is time consuming and expensive, however, cognitive deficits are not observable in routine neurological examinations and often hidden by more visible deficit (e.g. motor or sensory symptoms). Offering only a rough estimate of cognitive function (Rocca et al., 2015), cognitive function assessment in the context of the EDSS is not sufficient to specify cognitive impairment in patients with MS (Achiron et al., 2005). Other widely used screening methods for cognitive function, such as the Mini Mental State Examination (Marrie & Goldman, 2007) or the Multiple Sclerosis Neuropsychological Screening Questionnaire (Benedict et al., 2003) are insensitive to the cognitive profile of patients

with MS (Rocca et al., 2015). Furthermore, self-reported cognitive complaints are often confounded by mood and other subjective symptoms (Benedict & Zivadinov, 2011). Two validated cognitive test batteries have wide acceptance: the 45 min Brief Repeatable Battery of neuropsychological tests (Rao, 1991) and the 90 min Minimal Assessment of Cognitive Function in Multiple Sclerosis (MACFIMS) (Benedict, Cookfair, et al., 2006b). The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) (Langdon et al., 2012) has been lately developed and represents a short cognitive assessment (15min) for patients with MS, which is optimized for small centers with teams, which might not have training in neuropsychological tests. However, there is a need for the identification of additional neuropsychological assessment tools, which are particularly sensitive to MS-related cognitive deficits, are needed.
Apart from the cognitive abilities it is recommended to appropriately assess factors that potentially confound the interpretation of the neuropsychological data, including premorbid ability, fatigue and depression (Benedict et al., 2002). Moreover, sensory (e.g. visual) and motor disabilities as well as medication can lead to misinterpretation of cognitive performance and should therefore be accounted.

2.6 Neuroanatomical correlates of cognition

In the last three decades, many studies have tried to demonstrate an association between WM pathology and cognitive impairment. There was no doubt that fiber integrity in the WM, which is interconnecting association cortices, is essential for cognitive functioning. As a consequence, many studies aimed at correlating cognitive impairment WM matter lesion load/volume or at identifying specific locations of WM lesions and selective cognitive performance. For example, the disruption of WM tracts has long been regarded as an explanation for the slowed information processing speed. However, many researchers failed to make a clear conclusion regarding the interaction of WM lesions and cognitive performance (Foong et al., 1999; Franklin et al., 1989; Rao, Leo, Haughton, St Aubin-Faubert, & Bernardin, 1989; Swirsky-Sacchetti et al., 1992), concluding the severity of cognitive impairment in MS cannot be explained by WM lesions completely. The fact that the association between common neuroradiological markers (e.g. T2 lesion load) and clinical disability is weak is referred to as the 'clinico-radiological paradox' (Barkhof, 2002).
Recent technical developments have led to a further clarification of cognitive impairment in MS, highlighting the contribution of cortical lesions to cognitive impairment. Significant correlation between total cortical lesion load and global cognitive impairment index were found in a cross-sectional and longitudinal study.

In the former, cognitively impaired MS patients showed more cortical lesions and a higher cortical lesion volume than patients, which were cognitively preserved (M. Calabrese et al., 2009). In the longitudinal study, a high baseline volume of cortical lesions predicted worsening of cognitive performance (M. Calabrese et al., 2012). Only few studies assessed the association between lesion location and function. For instance, accumulation of cortical lesions in mesio-temporal areas is associated with impairments in episodic memory (Roosendaal et al., 2009).

Compared to lesion load, MRI markers of atrophy such as increased third ventricle width or reduced total white and grey matter volumes appear to be even more closely linked to cognitive decline and are consistently found to correlate more strongly with cognitive deficits (Sanfilipo, Benedict, Weinstock-Guttman, & Bakshi, 2006; Zivadinov et al., 2001). This is particularly true for the TVW, which is considered the best single MRI predictor for MS-related cognitive impairment (Benedict, Bruce, et al., 2006; Benedict et al., 2004). However, also global white and grey matter atrophy is related with multitude of cognitive impairment such as deficits in processing speed and working memory (Tekok-Kilic et al., 2007), verbal and nonverbal episodic memory (Benedict, Bruce, et al., 2006; Deluca et al., 2013), or decision making (Radomski et al., 2015). Furthermore, Calabrese and colleagues (2010) identified different patterns of cortical thickness in MS patients with and without cognitive impairment. Only a limited number of studies have assessed the association of regional brain atrophy and more specific cognitive impairment: For instance, bilateral hippocampal atrophy has been related to lowered episodic memory performance (Benedict, Ramasamy, Munschauer, Weinstock-Guttman, & Zivadinov, 2009), and focal cortical thinning in the bilateral fusiform gyrus to impaired processing of facial expression (Mike et al., 2013). In a very recent study, larger cortical volume in the supramarginal and superior temporal region of the right hemisphere was associated with visuo-spatial memory, and verbal memory to a larger cortical surface area in the lateral occipital, fusiform and inferior temporal region (Nygaard et al., 2015).

3 Aims

The rationale of the present work is to contribute to a better understanding of the underlying neural, in particular cortical structures responsible for the cognitive impairment in patients suffering from MS. The motivation for this main aim is based on previous research investigating the neuronal underpinnings of cognitive impairment in MS patients, which still shows a inconsistent and incomplete image. Furthermore, cognitive functioning is essential for being part of our - achievement and efficiency-oriented - society. Thus, with a better understanding of the responsible neural structures of cognitive functioning in MS, new prevention and therapy guidelines can be developed.

By reviewing the pertinent research of neural correlates of cognitive impairment, three research gaps emerged. Although the clinical importance of cortical pathology has been shown in the recent years, studies investigating its association to cognitive functioning are insufficient and many open questions are remaining. Second, despite the increasing amount of correlational studies between neuroanatomical alterations and broad defined cognitive dysfunction in MS, topological studies on specific cognitive dysfunctions in MS are very rare. And third, despite the identification of executive dysfunction in up to 20% of patients with MS and its devastating effects on patients' everyday life, studies examining these deficits are rare, compared to studies on mnestic deficits or slowing in cognitive processing speed. Moreover, random number generation, a test providing a sensitive measure of frontal executive function (Baddeley, Emslie, Kolodny, & Duncan, 1998; Brugger, Monsch, Salmon, & Butters, 1996), has never been applied in MS patients and its anatomical correlates are unexplored.

Based on these gaps, the following research questions arose:

1. Do patients with and without cortical lesions differ regarding cognitive, demographical and neurostructural characteristics?

2. What are the global and topological neural underpinnings of executive dysfunction in MS patients?

3. Is the randomization performance in MS patients different than the one from healthy controls and what are the neuroanatomical correlates of randomization performance?

This dissertation, which aligned a cross-sectional study design and used the methods of structural MRI, includes three studies, which try to give an answers to the above mentioned three research questions (Chapter 5). The first study focussed on the differences of disease-characteristc patterns between MS patients with and without DIR-hyperintense cortex-involving lesions. The second and the third study focussed more on specific cognitive issues, namely on executive dysfunctions in MS. Thus, whereas in the second study, global and topological correlates of executive dysfunctions in MS patients were investigated, the third study examined - for the first time - the performance of MS patients in a random generation task.

4 Methods

This section refers to the methods used in the three studies included in this dissertation. A brief overview is given here, whereas further details on the study-specific samples and methods will be discussed in the empirical part (Chapter 5).

4.1 Participants

The study samples of the three studies are based on the same sample of subjects. Fifty-one patients with a definite diagnosis of MS according to the McDonald 2010 criteria (Polman et al., 2011) and the most frequent, relapsing-remitting course were recruited at the Multiple Sclerosis Centre of the University Hospital of Zurich. Three patients had to be excluded from further analyses due to big lesion load causing problems in the brain tissue segmentation. Furthermore, 48 age-, gender-, handedness-, and education-matched healthy controls (HC) without previous or present history of neurological or psychiatric dysfunction were included. All participants had to satisfy the following inclusion criteria: native german speaker (Swiss German or other variant), minimal education of an apprenticeship and no current or past substance abuse. Furthermore, based on most recent clinical reports and subjective complaints, the following exclusion criteria for the patient group were applied: a) relapse or steroid-treatment during the last two months, b) current or past neurological disorder in addition to MS, c) psychiatric disorder apart from MS-related depressive mood state, d) a reading-relevant visual acuity deficit, and e) a writing- and/or drawing-relevant upper limb sensorimotor impairment of the dominant hand. Forty-seven patients (98%) were treated with an immunomodulatory drug - 32 with natalizumab (67%), ten with beta-interferon (21%), three with fingolimod (6%), one with glatiramer acetat (2%), one with dimethylfumarat (2%) and one patient had no disease-modifying therapy (2%). Control participants received financial compensation for their attendance. Table 4.1 gives an overview of the two groups.

Table 4.1: Demographics of the patient and the control group

	Control group	**MS group**	***t***	***Z***	χ^2	***p***
N	48	48				
Age	M = 38.02	M = 38.79	.398			.691
	SD = 9.6	SD = 9.3				
	Range = 21-57	Range = 18-58				
Years of Education	M = 14.58	M = 14.44		-.846		.398
(years)	SD = 2.5	SD = 3.05				
	Range = 11-20	Range = 11-22				
Sex					.00	1.00
female	37	37				
male	11	11				
Handedness					.447	.506
left	6	4				
right	42	44				

Note. Two-tailed tests.

4.2 Cognitive assessment

An extensive battery of neuropsychological tests, lasting about two hours, was administered to each subject, assessing a variety of cognitive functions such as executive functions, attention, learning and memory, visuospatial functions and premorbid intelligence. The selection of tests is based on psychometric properties and on recommendations regarding the cognitive domains that should be examined in MS patients (Benedict et al., 2004). Except of one (Mental Dice Task), all tests are standardized, validated and provide age-related norms. Additionally, three questionnaires were used to evaluate self-reported fatigue, depression and quality of life. Table 4.2 gives an overview of the neuropsychological tests and the questionnaires.

Table 4.2: Neuropsychological tests and questionnaires included in the neuropsychological examination.

Neuropsychological function	**Neuropsychological test**	**Norm.** $N =$	**Reference**
	Executive functions		
Working memory	Tests of Attentional Performance, working memory, level 3	322	Zimmermann and Fimm (2007)
Verbal fluency	Regensburger verbal fluency test	634	Aschenbrenner, Tucha, and Lange (2000)
Design fluency	HAMASCH-5-point test	184	Haid et al. (2002)
Interference control	Delis-Kaplan Executive Function System, colour-word interference condition	1750	Delis, Kaplan, and Kramer (2001)
Cognitive flexibility	Delis-Kaplan Executive Function System, colour-word set-shifting condition	1750	Delis et al. (2001)
Cognitive flexibility / Randomization performance	Mental Dice Task		Brugger, Milicevic, Regard, and Cook (1993)
	Attentional functions		
Alertness (tonic & phasic)	Tests of Attentional Performance , alertness	604	Zimmermann and Fimm (2007)
Selective Alertness	Tests of Attentional Performance, Go/NoGo	417	Zimmermann and Fimm (2007)
Information processing speed	Delis-Kaplan Executive Function System, colour naming condition/word reading test (read & colour condition)	1750	Delis et al. (2001)
	Learning & memory		
Verbal memory span	Wechsler Memory Scale - revised, verbal span	201	Härting et al. (2000)
Visual memory span	Wechsler Memory Scale - revised, visual span	201	Härting et al. (2000)
Verbal learning	Verbal learning memory test, five learning trials	500	Helmstaedter, Lendt, and Lux (2001)
Verbal recall	Verbal learning memory test, short and long delay free recall	500	Helmstaedter et al. (2001)
Verbal recognition	Verbal learning memory test	500	Helmstaedter et al. (2001)
Visual recall	Rey Complex Figure Test, short & long delay free recall	601	Meyers and Meyers (1995)
Visual recognition	Rey Complex Figure Test, recognition	601	Meyers and Meyers (1995)
	Visuoconstructive functions		
Visuospatial & -constructive performance	Rey Complex Figure Test, copy	601	Meyers and Meyers (1995)
	Cognitive reserve		
Premorbid intellectual level	Verbal short intelligence test	1117	Anger, Mertesdorf, Wegner, and Wülfing (1998)
Premorbid intellectual level	Multiple Choice Word Test-B	1952	Lehrl (2005)
	Questionnaires		
Depression	General Depression Scale	1298	Hautzinger and Bailer (1992)
Physical and cognitive fatigue	Würzburger Fatigue Inventory for MS	161	Flachenecker, König, Meissner, and Müller (2008)
Quality of life	Short Form Health Survey Questionnaire	2914	Bullinger and Kirchberger (1998)

4.3 Volumetric MR Imaging

A number of computer-assisted post-processing techniques for measuring white and grey matter MS lesion load as well as brain atrophy have been developed to provide quantitative means for determining the extent of disease abnormality. In the following sections, the technique to quantify lesion load and several atrophy parameters will be introduced shortly.

4.3.1 How to quantify lesion load in Multiple Sclerosis?

Quantitative characterization of MS lesion load is of central importance to clinical studies investigating the impact of lesions on cognitive or physical abilities. Many manual, semi-automated and automated segmentation techniques have been proposed to quantify total lesion volume as well as the total number of lesions (Lladó et al., 2012). Despite recent technical progress, due to the heterogeneous intensities among different MRI images, automated segmentation of MS lesions is a challenging task. Fully automated methods are often error prone and less accurate than the manual segmentation of MS lesion. The latter, in contrast, is known to be time-consuming and shows a high inter-oberserver variability (Lladó et al., 2012). There is not yet a specific automated lesion segmentation approach robust enough to emerge as a standard for clinical and research practice. Thus, in order to quantify the lesion volume in the studies included in this thesis, the lesions were manually delineated with MRIcron (http://sph.sc.edu/comd/rorden/mricron). Once all visible lesions of each patient were marked on individual three-dimensional scans, the total lesion volume and number was extracted and used for further analyses. In order to perform topological lesion analyses, normalization of the individual brains to the standard space of Montreal Neurological Institute with SPM8 (http://www.fil.ion.ucl.ac.uk/spm) was needed. The normalized lesion plots were then mapped on a template brain with MRIcron to create lesion overlap plots. MRIcron comprises a non-parametric mapping (NPM) software, which allows topological analyses and which is designed to relate lesion location to behavioral performance (Rorden, Karnath, & Bonilha, 2007). With this voxelbased approach, it is possible to elucidate structure-function relationships. Thus, in order to calculate the relationship between the location of the lesions and the behavioral performance voxel-based lesion symptom mapping (VLSM) was performed, which is a voxel-based statistical method that allows a correlation between behavioural measures and lesions on a voxel-by-voxel basis (Kimberg, Coslett, & Schwartz, 2007). VLSM was performed in Study 2.

4.3.2 Brain atrophy

In addition to lesions, atrophy is another imaging hallmark of MS. The improvement in post-processing methods allows to quantify the extent of tissue loss in white and grey matter as well as the distribution of atrophy at a regional level. Thus, beside the quantification of lesion load, central, whole-brain and cortical atrophy were assessed.

Whole-brain atrophy

Brain parenchymal fraction (BPF) was used as a normalized measurement of whole-brain atrophy. BPF was calculated as the ration of brain parenchymal tissue volume to the total volume contained within the brain surface contour (total intracranial volume). The calculation was performed with Jim software (Xinapse Systems Ltd., Northants (UK); http://www.xinapse.com).

Central atrophy

To assess central atrophy, third ventricular width was measured from the FLAIR images by applying the procedure specified by Benedict and colleagues (2006). Accordingly, a line was drawn through the long axis of the third ventricle parallel to the interhemispheric fissure, where the ventricle was most visible. Then a second line, perpendicular to the first one, was drawn through the midpoint of the first line in order to measure (in millimeters) the width of ventricle, which was further used for the statistical analyses. The calculation was performed with Jim software (Xinapse Systems Ltd., Northants (UK); http://www.xinapse.com).

Cortical atrophy

Cortical thickness evaluation - representing a measurement of cortical atrophy - was performed by means of Freesurfer image analysis suite, which is documented and freely available online (http://surfer.nmr.mgh.harvard.edu). The procedure for the measurement of cortical thickness by Freesurfer has been validated against histological analysis (Rosas et al., 2002) and manual measurements (Kuperberg et al., 2003). For technical details of the analysis pipeline I refer to prior publications validating and describing the procedure (Dale & Sereno, 1993; Dale, Fischl, & Sereno, 1999; Fischl & Dale, 2000), as it would go beyond the scope of this thesis. However, a short summary is given here: The cortical thickness is defined as the shortest distance between the pial (the border between GM and CSF) and the white matter (the interface between white and grey matter) surface (Figure 4.1). Freesurfer's surface-based analysis (SBM) reconstructs models of the two surfaces

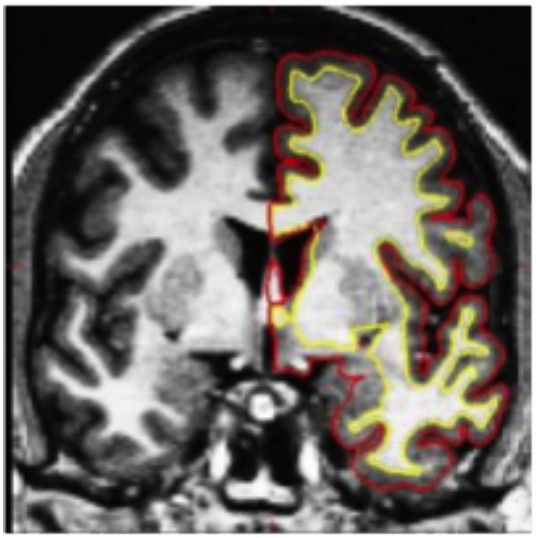

Figure 4.1: Brain with the two models of the pial (red) and white matter (yellow) surfaces, reconstructed by Freesurfer.

in each subject's native space by a set of automated tools. Briefly, this processing includes removal of non-brain tissue, registration to the Talairach space, intensity normalization and tessellation. Furthermore, the surfaces can be parcellated into gyral and sulcal regions of interest (Desikan et al., 2006). Once these surfaces are known, the evaluation of cortical thickness at each point on the cortex becomes possible. Metrics resulting from this approach are fairly straightforward to interpret, as they clearly represent a geometrical property of the cortex and are stated in standard units (e.g. cortical thickness in mm), which makes statistical interpretation easy. In order to do group analyses, a nonrigid high-dimensional spherical averaging over all patients, respectively controls was needed. With this method, it is possible to accurately match morphological homologous cortical locations among participants. A cortical surface-based atlas is defined, which is based on average folding pattern of all subjects mapped to a sphere. Thus, the surfaces from individuals can be aligned with this atlas, providing a point-to-point correspondence between the subjects. Once all surfaces are registered onto a standard template surface, vertex-wise analyses can be done.

5 Empirical part

This doctoral thesis is based on three first authorship articles:

Study 1
The relevance of cortical lesions in patients with Multiple Sclerosis

Study 2
Cortical thinning in the anterior cingulate cortex predicts Multiple Sclerosis patients' fluency performance in a lateralized way

Study 3
Random number generation deficits in patients with Multiple Sclerosis: Characteristics and neural correlates

5.1 Study 1

The relevance of cortical lesions in patients with Multiple Sclerosis

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5.1.1 Abstract

Background: Recent studies suggest that cortical lesions in multiple sclerosis (MS) substantially contribute to clinical disease severity. The present study aimed at investigating clinical, neuroanatomical, and cognitive correlates of these cortical lesions with a novel approach, i.e. by comparing two samples of relapsing-remitting multiple sclerosis (RRMS) patients, one group with and the other without cortical lesions.
Methods: High-resolution structural MRI was acquired from 42 RRMS patients and 43 controls (HC). The patient group was dichotomized based on the presence versus absence of DIR-hyperintense cortex-involving lesions, resulting in a cortical lesion group (CL, n = 32) and a non-cortical lesion group (nCL, n =10). Cognitive functioning was assessed in all participants with a comprehensive neuropsychological battery, covering mnestic, executive, and attentional functions.
Results: Highest densities of cortical lesions in the CL group were observed in the bilateral parahippocampal gyrus. Relative to HC, patients with cortical lesions - but not those without - showed significant global cortical thinning and mnestic deficits. The two patient groups did not differ from each other regarding demographic and basic disease characteristics such as EDSS scores.
Conclusion: The appearance of cortical lesions in MS patients is associated with cortical thinning as well as mnestic deficits, which might be key characteristics of a 'cortically dominant' MS subtype.

5.1.2 Background

First histological reports of demyelinated foci in the cerebral cortex of patients with multiple sclerosis (MS) date back to the beginning of the 20^{th} century (Dawson, 1916). However, the classical view of MS as a pure white matter pathology has not been overcome until the beginning of the 21^{st} century. Due to the introduction of more sensitive imaging techniques such as double inversion recovery (DIR), gray matter involvement in the pathology of multiple sclerosis is now well established (Geurts & Barkhof, 2008; M. Calabrese, Magliozzi, et al., 2015). However, enthusiasm for the use of DIR was somewhat decreased by the observation that it still can only detect a minority of cortical lesions in MS, when directly compared with post-mortem histopathological findings (Seewann et al., 2012).
With regard to the functional relevance of cortical MS lesions, cross-sectional studies have shown that lesion number and volume are associated with physical (M. Calabrese et al., 2007) and cognitive impairment (M. Calabrese et al., 2009; Mike et al., 2011). However, these studies are not without limitations. For example, detailed cognitive investigation (M. Calabrese et al., 2009) or DIR sequences were lacking (Mike et al., 2011). The present study was aimed at investigating the clinical relevance of cortical lesions with a novel methodological approach, i.e. by dichotomizing patients based on the presence or absence of cortical lesions. The resulting two subgroups were compared with regard to demography, cognition, fatigue, affective mood state, and several other established MRI markers of disease severity, for example T2 lesion load, third ventricle width, and global cortical thinning.

5.1.3 Methods

Participants

Forty-two patients with a diagnosis of relapsing-remitting multiple sclerosis (RRMS) according to the McDonald 2010 criteria (Polman et al., 2011) were recruited at the Multiple Sclerosis Centre of the University Hospital of Zurich. Patients with at least one DIR-hyperintense cortex-involving lesion were assigned to the cortical lesion group (CL group, n = 32), the remaining patients formed the non-cortical lesion group (nCL group, n = 10). All patients received immunomodulatory treatment - 30 with natalizumab, seven with beta-interferons, three with fingolimod, one with glatiramer acetat and one with dimethylfumarate. Exclusion criteria were a relapse or steroid-treatment during the last two months, current or past neurological disorder in addition to multiple sclerosis, and psychiatric disorders apart from a MS-related depressive mood state. Moreover, none of the patients was af-

fected by severe visual deficits or upper limb sensorimotor impairment that could hinder cognitive test performance. Furthermore, 43 age-, gender-, handedness- and education-matched healthy control persons (HC) without previous or present history of neurological or psychiatric diseases were included. Controls received financial compensation for their attendance. The study was approved by the local Ethical Committee, and written informed consent was obtained from all subjects.

MRI data acquisition

The MR scan was performed within one month of the neurological and neuropsychological examinations. All images were acquired using a 1.5-T scanner (Siemens Magnetom AvantoTM) equipped with a SQ-engine gradient (45m/T/m @ 200 T/m/s) using a dedicated 32-channel head coil. No hardware upgrades of the scanner occurred during the study period. The following sequences were obtained from all subjects: (1) 3D Double Inversion Recovery sequence (DIR) (voxel size =1.5 x 1.5 x 1.5 mm, slice thickness = 1.5 mm, repetition time = 7500ms, echo time = 308ms); (2) 3D T1-weighted MPRAGE (voxel size = 1 x 1 x 1 mm, slice thickness = 1 mm, repetition time = 2420 ms, echo time = 4.18 ms) and (3) a 3D FLAIR (voxel size = 0.9 x 0.9 x 2.0 mm, slice thickness = 2 mm, repetition time = 5000 ms, echo time = 342 ms).

MRI post-processing and statistical analysis

The classification of cortical lesions was conducted according to the consensus recommendations of Geurts and colleagues (Geurts et al., 2011). Consequently, cortical lesions were defined as those lesions appearing hyperintense on DIR images compared to surrounding normal-appearing gray matter, entirely or partly located in the cortical gray matter and occupying at least three voxels. Juxtacortical lesions (lesions not entering, but neighboring the cortical mantle) were not scored. DIR-hyperintense lesions were identified and manually delineated with MRIcron (http://sph.sc.edu/comd/rorden/mricron), which was further used to measure total cortical lesion volume. An experienced rater, supervised by a neuroradiologist, assessed all images. The same procedure was applied to FLAIR images in order to identify FLAIR-hyperintense lesions. We used Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/) to co-register and normalize the individual lesion maps to MNI (Montreal Neurological Institute) standard space. Normalized lesions were then mapped with MRIcron on a template brain to create lesion overlap plots. Central brain atrophy was examined by measuring the width of the third ventricle (TVW) according to the procedure proposed by Benedict and colleagues (Benedict, Bruce, et al., 2006). Brain parenchymal fraction (BPF),

calculated as the ratio of brain parenchymal tissue volume to the total intracranial volume, was used as a measurement of whole-brain atrophy. Cortical thickness evaluation was performed with the semi-automated Freesurfer image analysis suite based on MPRAGE images, which is documented and freely available online (http://surfer.nmr.mgh.harvward.edu). Further information and technical details of these procedures are described in prior publications (Fischl, Sereno, & Dale, 1999; Dale et al., 1999). To detect possible misclassifications of white and gray matter due to multiple sclerosis lesions, all images were visually inspected after the white/gray matter segmentation. In two patients, a semi-automated correction of topological defects was required. We used the manual procedure of control points, which is implemented in the Freesurfer software package.

Clinical and neuropsychological assessment

All patients underwent neurological status examination, including a rating of the Expanded Disability Status Scale (EDSS). Cognitive functions were assessed with a comprehensive battery of validated and standardized neuropsychological tests. To minimize the issue of multiple statistical testing, composite index scores were computed for mnestic, executive and attentional functions by averaging z-scores for all subtests of the corresponding function (La Joie et al., 2014). An overview of the indices is shown in supplemental Table 5.2 (see Supplemental material in Section 5.1.7). The mnestic index score was derived from the delayed free recall and recognition of a 15-item word list (Helmstaedter et al., 2001) as well as the delayed free recall and recognition of a previously copied complex geometric figure (Meyers & Meyers, 1995). Phonemic-verbal (Aschenbrenner et al., 2000) and figural fluency (Haid et al., 2002), response inhibition (Delis et al., 2001) and cognitive flexibility (Delis et al., 2001) formed the executive index. The attentional index was based on processing speed during color naming (Delis et al., 2001) as well as on reaction times from tasks measuring alertness and selective attention (Zimmermann & Fimm, 2007). Moreover, participants had to complete a German version (Hautzinger & Bailer, 1992) of the CES-D Depression questionnaire (Radloff, 1977) and the Würzburg Fatigue Inventory (WEIMuS) (Flachenecker et al., 2008) to self-rate depressive symptoms as well as fatigue during the last week. Similar to other studies (Sumowski et al., 2014), cognitive reserve was examined with vocabulary knowledge (multiple choice word test) (Lehrl, 2005).

Statistical analysis

Statistical analyses were performed with SPSS (IBM, Chicago, USA, Version, 21.0, http://spss.com). Unless otherwise stated, a p-level below 5% was considered

Table 5.1: Demographic and disease characteristics of multiple sclerosis patients and healthy controls.

	nCL group (n = 10) Mean (SD)	CL group (n = 32) Mean (SD)	Controls (n = 43) Mean (SD)	Test	p-value
Age, years	32.6 (9.98)	38.22 (7.2)	36.1 (8.29)	ANOVA	0.155
Education, years	15.5 (3.1)	14.45 (3.2)	14.58 (2.4)	KW	0.408
EDSS	2.0 (1.8)	2.8 (1.8)	-	MW-U	0.174
Age at diagnosis, years	26.7 (9.7)	30.65 (7.6)	-	t-test	0.185
Disease duration, years	65.5 (59.6)	85.31 (68.9)	-	MW-U	0.570

Abbreviations: nCL = patients without cortical lesions; CL = patients with cortical lesions; one-way ANOVA = one-way analysis of variance; EDSS = Expanded Disability Status Scale; KW = Kruskal-Wallis; MW-U = Mann-Whitney U

statistically significant. Assumptions for normality were tested for all continuous data with Kolmogorov-Smirnov tests. In case of normally distributed variables, a multivariate ANOVA was used to compare groups. Bonferroni correction was applied for post-hoc analyses. When variables were not normally distributed, non-parametric tests were applied (Mann-Whitney U, Kruskal-Wallis).

5.1.4 Results

Demographic and clinical variables

Demographic and clinical characteristics of the three groups are reported in Table 5.1. No between-group differences were found with regard to age, education, and gender. Furthermore, EDSS, age at diagnosis, and disease duration did not differ significantly between CL and nCL patients.

MRI markers

We detected cortical lesions in 32 of 42 patients (76%). The highest occurrence of cortical lesions was found bilaterally in the parahippocampal gyrus (Figure 5.1). Twenty-five percent of the CL patients showed at least one cortical lesion in this region.

An overview of the atrophy measurements of the three groups is given in Figure 5.2. The three groups differed significantly from one another with regard to BPF ($F(2) = 22.4$, $p < 0.001$), TVW ($H(2) = 20.9$, $p < 0.001$) and cortical thickness ($F(2) = 11.6$, $p < 0.001$). Bonferroni-corrected post-hoc analyses revealed a reduction of BPF in CL patients relative to HC ($p < 0.001$) as well as an enlarged TVW ($p < 0.001$). In contrast, the BPF and TVW values of nCL patients did not differ from those of CL patients or controls. Furthermore, Bonferroni-corrected post-hoc analyses revealed a reduction of global cortical thickness in CL patients compared

to HC (p<0.001) and nCL (p = 0.029) patients, while HC and nCL patients did not differ from one another. Regarding the FLAIR-hyperintense lesion volume no significant difference (p = 0.064) was observed between the two patient groups.

Cognitive test results, fatigue and depression questionnaires

The attentional index did not differ between the three groups (F(2) = 1.207, p = 0.304). In contrast, a group effect was observed for the executive index (H(2) = 22.476, p<0.001). Both patient groups showed executive dysfunction compared to HC (CL vs. HC: Z(2) = -4.272, p<0.001; nCL vs. HC: Z(2) = -3.197, p ¡ 0.001), but did not differ from one another (Z(2) = -0.251, p = 0.805). Furthermore, there was a main group effect for mnestic functions (F(2) = 7.667, p<0.001). Bonferroni-corrected post-hoc analyses revealed that the CL group performed significantly worse in memory tests than the nCL (p = 0.031) as well as the HC group (p<0.001), whereas the nCL patients did not differ from the HC in this regard. Figure 5.3 depicts the three cognitive indices and corresponding differences between groups. According to supplemental analyses (summarized in supplemental Table 5.3, see Supplemental material in Section 5.1.7) of the individual subtests of the indices, differences between the patient groups were most strongly pronounced in figural recognition. No main group effect was seen for depression (H(2) = 5.634, p = 0.060), but both patient groups showed significantly elevated fatigue scores compared with HC (CL vs. HC: Z(2) = -2.319, p = 0.020; nCL vs. HC: Z(2) = -2.102, p = 0.036), but did not differ from one another in this regard (Z(2) = -0.445, p = 0.673).

5.1.5 Discussion

The impact of gray matter pathology, in particular that of gray matter lesions, on cognitive and physical functioning in MS patients has been discussed for many years. Here we highlight the clinical relevance of DIR-hyperintense cortical lesions in patients with RRMS. The most intriguing finding of the present study was that patients with - compared to those without visible cortical lesions - differed from each other in global cortical thickness and mnestic functions, whereas no differences between these two patient groups were observed regarding EDSS, age, age at diagnosis, disease duration, or non-mnestic cognitive functions. Moreover, patients without cortical lesions showed normal cortical thickness and mnestic functions, when compared with a group of healthy controls. That a reduction of cortical thickness can occur in MS (e.g. Sailer et al., 2003)- and that this thinning is related to global (e.g. M. Calabrese, Rinaldi, et al., 2010) and even specific cognitive impairment (Mike et al., 2013; Geisseler et al., 2016) - has been found in previous

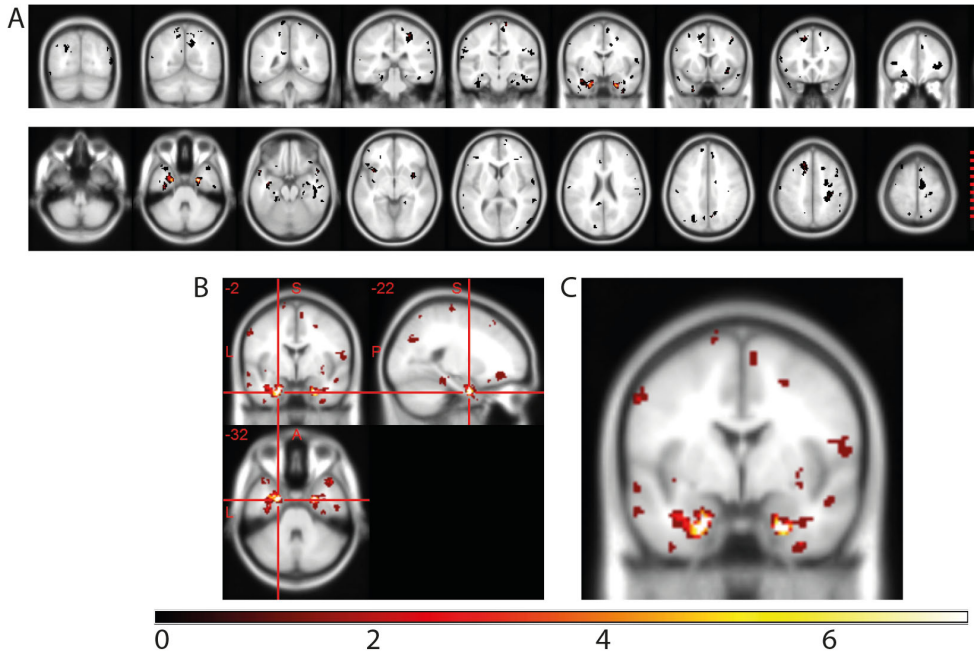

Figure 5.1: Spatial distribution of cortex-involving lesions in the CL patient group. Overlap plot based on all normalized cortex-involving lesions found in all CL patients. Lesion frequency across the sample is displayed for every depicted voxel. The bar indicates the number of patients showing damage to a particular voxel. A) Axial and coronal views of lesion frequency. B) and C) Highest lesion overlap was found in the bilateral parahippocampal gyrus (MNI coordinates in B). Image orientation follows the radiological convention (right on left side).

studies. Similar to a recent finding (M. Calabrese, Reynolds, et al., 2015), we show an association between MS-related cortical thinning and the presence of cortical MS lesions. It is known that gray matter pathology involves both inflammatory and degenerative mechanisms, but the relationship between the two remains unclear (M. Calabrese, Magliozzi, et al., 2015). Gray matter atrophy might be the final step of several pathological processes, which could include cortical demyelination but also retrograde degeneration secondary to white matter lesions and, perhaps, primary neurodegeneration (Geurts et al., 2012).

We detected DIR-hyperintense cortical lesions in 76% of our RRMS patients, supporting the notion of a high prevalence of these lesions (M. Calabrese et al., 2007). In further agreement with previous findings (M. Calabrese, Favaretto, Martini, & Gallo, 2013), we observed an uneven spatial distribution of cortical lesions over the cerebral cortex, with a prominent accumulation in memory-relevant mesiotemporal regions, particularly in the bilateral parahippocampal gyrus. While the importance of the hippocampus for memory function is known since the classical description of the patient H.M. in 1957 (Scoville & Milner, 1957), parahippocampal involvement in memory functions was recognized only two decades ago (Zola-Morgan, Squire, Amaral, & Suzuki, 1989). Squire and Zola-Morgen (1991) identified the anatomical components of what is termed the medial temporal memory system.

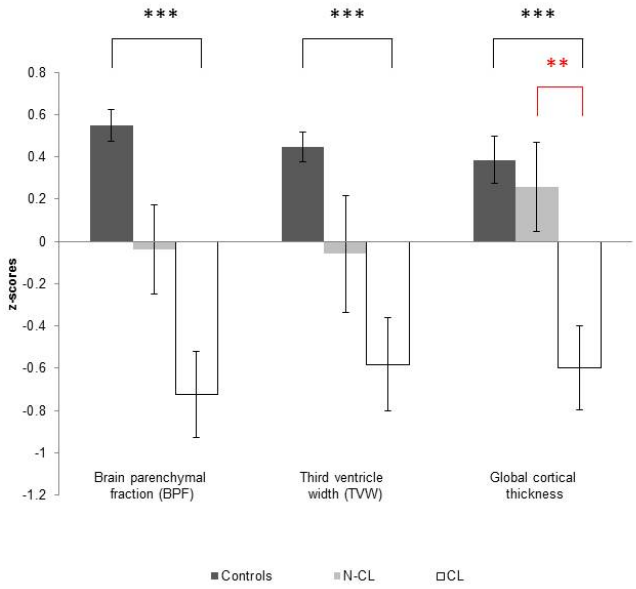

Figure 5.2: Bars depict mean z-scores (and standard errors) of patients with (CL) and without cortex-involving (nCL) lesions as well as of healthy controls (HC). Note that the two patient groups differed only in global cortical thickness.

By now it is well known that bilateral damage to the medial temporal lobe causes severe learning and memory impairments. This relationship has also been shown in MS patients. Learning and memory is the most frequently disrupted cognitive domain in MS, reported in 40-60% of patients (Rogers & Panegyres, 2007). Moreover, Coebergh et al. (2010) described a patient with acute memory impairment, associated with hippocampal and cortical lesions. Cortical lesions were also associated with cognitive decline in a group of 13 MS patients (Roosendaal et al., 2009). In this study, a significant correlation between hippocampal lesion load and visuospatial memory was observed. Based on the present and previous findings, we thus conclude that mesiotemporal cortical lesions are highly prevalent in RRMS patients and play a crucial role in the development of mnestic dysfunction.
The association we found between memory impairment and both cortical thinning as well as cortical lesions seems particularly intriguing: One might speculate that mnestic dysfunction in MS patients could indicate cortical involvement of the MS pathology in general. Related to this assumption, a rarely occurring variant of so-called 'cortical MS' has been described in previous studies (Zarei, Chandran, Compston, & Hodges, 2003; Zarei, 2006). The condition was characterized by predominant presence of neurobehavioral symptoms (e.g. depression, apathy) and neuropsychological deficits (e.g. agraphia, anomia) suggesting cortical dys-

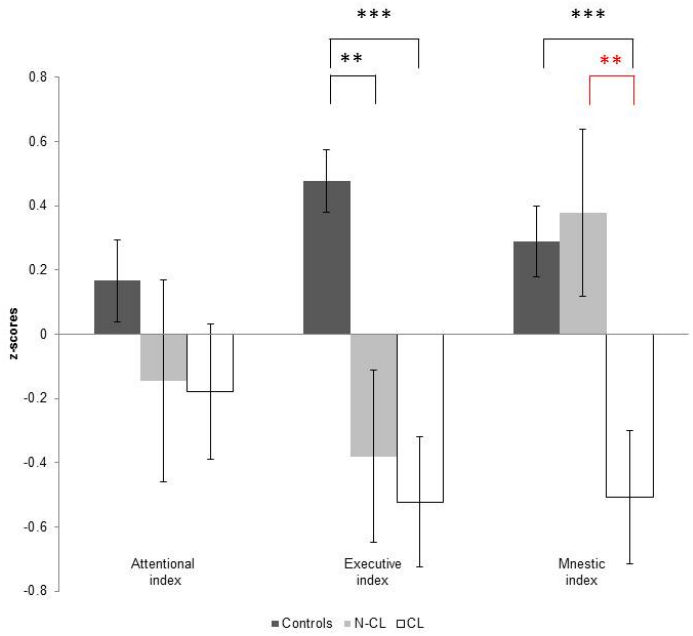

Figure 5.3: Bars show mean z-scores (and standard errors) of patients with (CL) and without cortex-involving (nCL) lesions as well as of healthy controls (HC). Note that the two patient groups differed only in mnestic functioning.

function. Our patient group with cortical involvement may reflect an incomplete variant of such cortical MS, as they showed distinct mnestic deficits in association with cortical involvement, but no other cortical symptoms such for example depression. Moreover, we found no patient with cortical lesions in the absence of subcortical lesions. In all patients, including those of the CL group, the majority of MS lesions was located subcortically. Taking this into account, we here propose that our CL group may represent a 'cortically dominant' subtype of MS. In these patients, pathophysiological processes might be different from those of patients without cortical involvement.

Much research and clinical development in MS has focused on the inflammatory mechanisms of the disease. Meanwhile, multiple disease-modifying drugs (DMD) are available that target the inflammatory pathology of MS, in particular the development of new white matter lesions (Barkhof, Calabresi, Miller, & Reingold, 2009). A recent study demonstrated that DMD - in particular IFN β -1a and glatiramer acetate - can reduce the accumulation of cortical lesions too (Rinaldi et al., 2015). In addition, Filippi and colleagues (Filippi et al., 2010) showed that the presence of at least one cortical lesion is associated with a high risk of conversion from clinically isolated syndrome (CIS) to definite MS within a short period. Together with our results, these findings highlight the relevance of cortical lesions

as a “target” in the development of new DMD, and to include cortical lesions as a primary outcome variable in disease and treatment monitoring.

5.1.6 Conclusion

In conclusion, the occurrence of cortical lesions in MS is clinically relevant insofar as it is associated with neurodegenerative cortical thinning and mnestic dysfunction. Although with today's imaging techniques, it is only possible to visualize the 'tip of the iceberg' of cortical MS lesions (Seewann et al., 2012, 2011), further progress in detection algorithms can be expected and will likely improve our understanding of MS pathology, symptoms, and treatment.

5.1.7 Supplemental material

Table 5.2: Neuropsychological tests building the three indices

Index	Cognitive function	Neuropsychological test	Variables
Mnestic Index			
	Verbal recall	Verbal learning memory test (VLMT)	Short-delay free recall (number of hits)
	Verbal recognition	Verbal learning memory test (VLMT)	Recognition (number of hits)
	Figural recall	Rey Complex Figure Test (RCFT)	Short-delay free recall (number of hits)
	Figural recognition	Rey Complex Figure Test (RCFT)	Recognition (number of hits)
Executive index			
	Verbal fluency	Regensburger verbal fluency test (RWT)	Number of hits
	Figural fluency	HAMASCH-5-point test	Number of hits
	Response inhibition	Delis-Kaplan Executive Function System, color word interference condition	Time to completion
	Cognitive flexibility	Delis-Kaplan Executive Function System, switching condition	Time to completion
Attention index			
	Tonic alertness	Tests of Attentional Performance (TAP) battery, alertness test	Median reaction time
	Phasic alertness	Tests of Attentional Performance (TAP) battery, alertness test	Median reaction time
	Selective attention	Tests of Attentional Performance (TAP) battery, Go-NoGo Test	Median reaction time
	Information processing speed	Delis-Kaplan Executive Function System, color naming condition	Time to completion

Table 5.3: Detailed analyses of attentional, mnestic and executive functions (z-scores)

	Controls (n = 43), mean z-score	nCL (n = 10), mean z-score	CL (n = 32), mean z-score	Controls vs. N-CL p-value	Controls vs. CL p-value	N-CL vs. CL p-value
			Mnestic functions			
verbal free recall	0.3367	0.2206	-0.5231	0.757	0.006**	0.138
figural free recall	0.177	0.2571	-0.3181	0.891	0.026	0.146
verbal recognition	0.319	0.1707	-0.4757	0.194	<0.001**	0.192
figural recognition	0.1842	0.4706	-0.3946	0.322	0.018*	0.016*
			Executive functions			
verbal fluency[a]	0.4708	-0.3087	-0.5355	0.43	<0.001**	0.999
figural fluency[a]	0.5531	-0.3462	-0.6344	0.005**	<0.001**	0.999
response inhibition	0.25	-0.0555	-0.3178	0.145	0.037	0.988
set shifting a	0.3014	-0.3263	-0.3026	0.026*	0.201	0.999
			Attentional functions			
tonic alertness	0.1457	-0.0153	-0.191	0.601	0.174	0.716
phasic alertness	0.1752	-0.0936	-0.2061	0.999	0.261	0.611
selective attention	0.0791	-0.043	-0.0861	0.602	0.884	0.631
information processing speed	0.3403	-0.3386	-0.35	0.031*	0.008**	0.782

Abbreviations: CL = patients with cortical lesions; nCL = patients without cortical lesions
Note. Unless otherwise noted statistical comparisons are based on Mann-Whitney U test.
* $p < 0.05$; ** $p < 0.01$.
[a] Because of normal distribution in both samples a t-test was performed

5.2 Study 2

Cortical thinning in the anterior cingulate cortex predicts Multiple Sclerosis patients' fluency performance in a lateralised manner

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5.2.1 Abstract

Cognitive impairment is as an important feature of Multiple Sclerosis (MS), and might be even more relevant to patients than mobility restrictions. Compared to the multitude of studies investigating memory deficits or basic cognitive slowing, executive dysfunction is a rarely studied cognitive domain in MS, and its neural correlates remain largely unexplored. Even rarer are topological studies on specific cognitive functions in MS. Here we used several structural MRI parameters – including cortical thinning and T2 lesion load – to investigate neural correlates of executive dysfunction, both on a global and a regional level by means of voxel- and vertex-wise analyses. Forty-eight patients with relapsing-remitting MS and 48 healthy controls participated in the study. Five executive functions were assessed, i.e. verbal and figural fluency, working memory, interference control and set shifting. Patients scored lower than controls in verbal and figural fluency only, and displayed widespread cortical thinning. On a global level, cortical thickness independently predicted verbal fluency performance, when controlling for lesion volume and central brain atrophy estimates. On a regional level, cortical thinning in the anterior cingulate region correlated with deficits in verbal and figural fluency and did so in a lateralised manner: Left-sided thinning was related to reduced verbal – but not figural – fluency, whereas the opposite pattern was observed for right- sided thinning. We conclude that executive dysfunction in MS patients can specifically affect verbal and figural fluency. The observed lateralised clinico-anatomical correlation has previously been described in brain-damaged patients with large focal lesions only, for example after stroke. Based on focal grey matter atrophy, we here show for the first time comparable lateralised findings in a white matter disease with widespread pathology.

Keywords

Multiple sclerosis, executive function, fluency, atrophy, cortical thinning

Abbreviation

EDSS = expanded disability status scale; FDR = false discovery rate; FLAIR = fluid attenuated inversion recovery; MPRAGE = magnetization prepared rapid gradient-echo imaging; MS = multiple sclerosis; TVW = third ventricle width; VLSM = voxel-lesion symptom mapping

5.2.2 Introduction

Multiple Sclerosis (MS) is a chronic demyelinating disease affecting all parts of the central nervous system. It used to be characterised primarily by its sensory, motor and visual symptoms. However, cognitive impairment is now recognised as an important feature of MS, prevalent in 43–70% of patients (Chiaravalloti & DeLuca, 2008). It might even be more relevant to patients than mobility restrictions (Amato et al., 2006). As MS has usually an early onset in young adult life (P. Calabrese, 2006), cognitive impairment significantly contributes to patients' disability status, is critical for working capacity, and thus negatively affects quality of life (Rao, Leo, Ellington, et al., 1991). Processing speed and episodic memory seem the most prominent cognitive features of MS (Chiaravalloti & DeLuca, 2008); however, MS patients often exhibit significant deficits in executive functions too (Drew et al., 2008). Although the latter can have devastating effects on patients' everyday life, their level of independence and societal costs (Amato et al., 2013), studies examining executive deficits in MS patients are rare (Foong et al., 1999; Henry & Beatty, 2006; Radomski et al., 2015) compared to the multitude of studies investigating memory deficits or basic cognitive slowing (Chiaravalloti & DeLuca, 2008; Rogers & Panegyres, 2007; Roth et al., 2015). Traditionally, MS has been thought of as a white matter disease, with focal demyelinating lesions in the white matter being the pathological hallmark. However, this view has been challenged in recent years. It is now known that grey matter areas including the cerebral cortex can also be affected (M. Calabrese et al., 2012), a finding demonstrated first in post-mortem analyses (Kidd et al., 1999). In addition to focal lesions, brains of MS patients sometimes show considerable atrophy (Barkhof, 2002), which affects not only white, but also grey matter structures. Atrophy can become manifest, for example, as widespread cortical thickness reduction, with a predominant involvement of temporal and frontal regions (M. Calabrese, Rinaldi, et al., 2010; Munger et al., 2004; Sailer et al., 2003).

Modern MRI techniques and post-processing methods assessing atrophy parameters contribute to the improvement in understanding the mechanisms responsible for physical and cognitive impairment in MS (Filippi & Rocca, 2010). During the last three decades, many studies have demonstrated an association between white matter pathology and cognitive impairment (e.g Rao et al., 1989). However, only modest correlations were found (e.g. Foong et al., 1997; Swirsky-Sacchetti et al., 1992), so that a majority of variance in cognitive performance remained unexplained. MRI markers of atrophy such as increased third ventricle width or reduced total white and grey matter volumes are consistently found to correlate more strongly with cognitive deficits (Amato et al., 2004; Benedict, Bruce, et al.,

2006) than white matter lesion load. In the last decade, grey matter pathology, i.e. cortical and deep grey matter atrophy and cortical lesion load, has been identified as a significant substrate of cognitive impairment, by showing particularly strong associations (e.g. Amato et al., 2004; M. Calabrese et al., 2009; Nielsen et al., 2013; Zivadinov et al., 2001).
Despite the huge amount of correlation studies between different structural alterations and cognitive dysfunction in MS, focal-topological studies on specific cognitive functions are rare. For example, deficits in sustained attention and working memory were related to lesions in bilateral frontal and parietal white matter (Sperling et al., 2001), and deficits in verbal learning to lesions in the left frontal lobe (Reuter & Fischl, 2011). With regard to atrophy, bilateral hippocampal atrophy has been related to impaired verbal learning (Sicotte et al., 2008).
The aim of the present study was to investigate executive dysfuncion in patients with MS and to analyse its relationship with both global and regional MRI markers. Our methodological focus was on cortical thinning. Only few previous studies have addressed the identification of cortical thinning in MS and its association to global cognitive impairment (M. Calabrese, Rinaldi, et al., 2010; Morgen et al., 2006; Tekok-Kilic et al., 2007). And to the best of our knowledge, only a single study so far investigated the impact of regional cortical thinning on specific cognitive disability, showing that focal thinning in the bilateral fusiform gyrus was related to impaired processing of facial expressions (Mike et al., 2013). Here we extend this approach by examining whether similar foci can be found for MS-related executive dysfunction.

5.2.3 Materials and methods

Participants

Forty-eight patients with a definite diagnosis of MS according to the McDonald 2010 criteria (Polman et al., 2011) and a relapsing-remitting course were recruited at the Multiple Sclerosis Centre of the University Hospital of Zurich. Forty-seven patients (98 %) were treated with an immunomodulatory drug - 32 with natalizumab, ten with beta-interferons, three with fingolimod, one with glatiramer acetat, one with dimethylfumarate, and one patient had no disease-modifying therapy. We applied the following exclusion criteria: a) relapse or steroid-treatment during the last two months, b) current or past neurological disorder in addition to multiple sclerosis, c) psychiatric disorder apart from multiple sclerosis-related depressive mood state, d) a reading-relevant visual acuity deficit, and e) a writing- and/or drawing-relevant upper limb sensorimotor impairment of the dominant hand. Furthermore, 48 age-, gender-, handedness-, and education-matched healthy

controls without previous or present history of neurological or psychiatric dysfunction were included. The study was approved by the regional Ethics Committee. All participants provided written informed consent. Control participants received financial compensation for their attendance.

Neuropsychological and neurological examination

Neuropsychological and neurological (only for the patient group) examinations were performed within one month of the MRI scan described below by experienced clinicians of the Multiple Sclerosis Centre. Five executive functions were examined. First, verbal-phonematic fluency was assessed with the Regensburger verbal fluency test (Aschenbrenner et al., 2000). Participants were required to generate as many words as possible beginning with the letter "s" in two minutes. Repetitions of word stems or deviations from test rules were regarded as errors; the number of correct answers was further analysed. The HAMASCH-five-points-test (Haid et al., 2002) was applied to assess figural fluency (Regard, Strauss, & Knapp, 1982). This test required participants to create as many unique designs as possible through connecting at least two dots of a five-dot pattern with straight lines within three minutes. The total number of unique designs entered further analyses. Furthermore, working memory was assessed by a two-back task(Zimmermann & Fimm, 2007), where the total number of errors (number of misses plus number of false positives) was further analysed. The Colour-Word-Interference subtest of the Delis–Kaplan Executive Function System (Delis et al., 2001) was used to investigate response inhibition and set shifting. In the interference condition, participants had to name the colour of the ink in which the word was printed – which is at conflict with the word meaning – while inhibiting the propensity to read the word. In the switching condition, participants had to irregularly alternate between reading the word and naming the ink, depending on the presence or absence of a box surrounding the word. Again, ink colour and word meaning were always at conflict with one another. Furthermore, a third condition of the D-KEFS colour-word interference test, i.e. the colour naming condition, served as a control parameter for basic information processing speed. As this study focuses on executive functions rather than on basic cognitive slowing, corrected time-to-completion indices were calculated with this latter parameter, according to the procedure specified by Delis and colleagues (2001). In other words, and concerning both interference control and set shifting, two variables entered further analyses, i.e. the number of correct answers and the corrected time-to-complete. Moreover, participants had to complete a German version (Hautzinger & Bailer, 1992) of the CES-D Depression questionnaire (Radloff, 1977) and the Würzburg Fatigue Inventory (WEIMuS) (Flachenecker et al., 2008) to self-assess depressive symptoms as well as cognitive

and physical fatigue during the last week. Similar to other studies (Sumowski et al., 2014), cognitive reserve was examined with vocabulary knowledge. In our study, the Multiple-Choice Word Test-B (Lehrl, 2005) was applied. Additionally, Expanded Disability Status Scale (EDSS) scores (Kurtzke, 1983) were obtained in all patients.

MR image acquisition protocol

All images were acquired with a neuro-optimised 1.5 Tesla MR scanner (Siemens Magnetom AvantoTM) equipped with a SQ-engine gradient (45m/T/m @ 200 T/m/s) using a dedicated 32-channel head coil. The sequences acquired in each subject included a T1-weighted Magnetisation Prepared Rapid Gradient Echo (MPRAGE; voxel size = 1 x 1 x 1 mm, slice thickness = 1 mm, repetition time = 2420 ms, echo time = 4.18 ms) and a T2-weighted Fluid Attenuated Inversion Recovery sequence (FLAIR; voxel size = 0.9 x 0.9 x 2.0 mm, slice thickness = 2 mm, repetition time = 5000 ms, echo time = 342 ms).

Image analysis

T2-hyperintense lesion volume: FLAIR-hyperintense lesions were identified and manually delineated with MRIcron (http://sph.sc.edu/comd/rorden/mricron), which was also used to measure total lesion volume. An experienced rater (TP), supervised by a neuroradiologist (BS), performed this lesion analysis. Statistical Parametric Mapping (SPM8, http://www.fil.ion.ucl.ac.uk/spm/) was used to co-register and normalize individual lesion maps to MNI (Montreal Neurological Institute and Hospital) standard space. Normalized lesion maps were then mapped on a template brain with MRIcron to create lesion overlap plots. Included in the MRIcron package, the non-parametric mapping software (Rorden et al., 2007) was used to analyze possible associations of behavioral performance with the spatial distribution of lesions, i.e. voxel-based lesion symptom mapping (VLSM).
Central atrophy: Central brain atrophy was examined by measuring the width of the third ventricle (TVW), applying the procedure specified by Benedict and colleagues (Benedict, Bruce, et al., 2006). Accordingly, a line was drawn through the long axis of the third ventricle parallel to the interhemispheric fissure on the axial MPRAGE slice on which the third ventricle was best visible. Then a second line, perpendicular to the first one, was drawn through the midpoint of the first line and measured with the Jim software (Xinapse Systems Ltd., Northants (UK); http://www. xinapse.com).
Cortical thickness analyses: Cortical thickness evaluation was performed with the Freesurfer image analysis suite, which is documented and freely available online

(http://surfer.nmr.mgh.harvard.edu). It is a set of automated processing procedures, requiring 3D MPRAGE MRI images and comprising skull-stripping, registration, intensity normalization, Talairach transformation, tissue segmentation and surface parcellation. Further information and technical details of the procedures are described in prior publications (Fischl et al., 1999). To detect possible misclassifications of white and grey matter, e.g. due to multiple sclerosis lesions, all images were visually inspected after the white/grey matter segmentation. In two patients, a semi-automated correction of topological defects was required by manually adding control points.

Statistical analysis

Statistical analyses of the demographic and cognitive data were per- formed in SPSS (IBM, Chicago, USA, Version, 21.0, http://spss.com). Un- less otherwise stated, a p-level below 5% was considered statistically significant. Assumptions for normality were tested for all continuous data with Kolmogorov–Smirnov tests, and parametric (t-test, Pearson correlation) or non-parametric tests (Mann–Whitney-U, Spearman rank correlation) were used when appropriate. Chi-Square tests were conducted to test for differences in frequency distributions between the two groups. Whole-brain vertex-wise statistical analyses of cortical thickness were performed with the Query Design Estimate Contrast (Qdec) module implemented in Freesurfer.

As a first step, we analysed significant group differences in demographic variables (age, cognitive reserve, gender, handedness), fatigue, depressive symptoms, global cortical thickness, third ventricle width, and executive functions. Further analyses were restricted to those executive functions for which significant impairments were observed. In order to analyse whole-brain vertex-wise differences in cortical thickness between the two groups a general linear model (GLM) was used. Key demographics (e.g. age, education) were not controlled for, as these did not statistically differ between groups. Group differences of the cortical thickness were separately calculated for each hemisphere. A false discovery rate (FDR) of 0.05 was used to control for multiple comparisons.

As a next step, we analysed the association of impaired executive functions with demographic and disease variables (age, disease duration, EDSS, education, fatigue, depressive symptoms) within the patient group. Any variable significantly associated with executive impairment were controlled for in subsequent analyses. In a third step, the relative contribution of global MRI variables on executive performance was examined by hierarchical regression analyses. Multicollinearity between MRI variables was assessed using the variance inflation factor. In a final step, the relationship between regional structural alterations and executive dys-

function was examined. A general linear model at each vertex point of the cortical surface model was used to investigate the correlation between areas of regional cortical thickness with executive impairment. Statistical surface maps showing significant correlations between cortical thinning and executive performance were generated by thresholding the images of t-statistics at a 0.05 significance level, corrected for FDR. For the VLSM we performed Brunner–Munzel tests (Brunner & Munzel, 2000) at a threshold of 5% FDR to identify lesioned voxels associated with executive deficits. Only voxels affected in at least two patients were considered for analysis. For all vertex- and voxel-wise analyses, only clusters with a continuous extent of 10 mm^2 were reported.

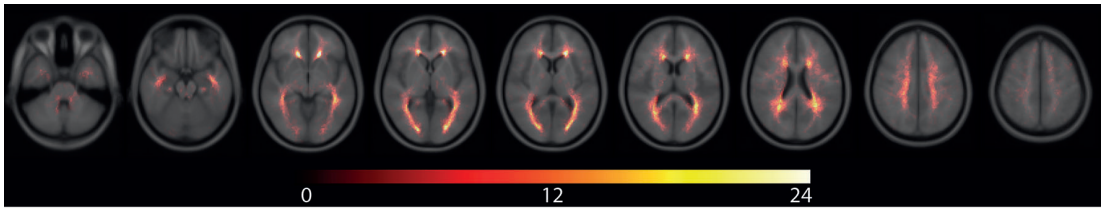

Figure 5.4: Spatial distribution of T2-hyperintense lesions: All normalised FLAIR-hyperintense lesions of all patients are shown in the lesion maps, illustrating pronounced periventricular accumulation. Image orientation follows the radiological convention (right on left side). The color bar indicates the number of overlapping lesions.

5.2.4 Results

Demographic, clinical and conventional MRI assessment

Age (p = 0.691), education (p = 0.398), gender (p = 0.999), and handedness (p = 0.506) did not significantly differ between controls and patients. An overview of the main demographic, clinical and MRI features of patients and controls is given in 5.4. Lesion probability maps are shown in Figure 5.4, displaying the expected accumulation (Rossi et al., 2012) of of T2-hyperintense lesions in periventricular areas.

Cognitive findings

Test and questionnaire results are displayed in Table 5.5. Cognitive reserve was nearly identical in the two groups. Performances in the working memory, response inhibition and set shifting tests did not significantly differ between controls and patients (all p >0.05). However, differences were found in the number of items generated during the verbal and figural fluency tasks (both p <0.001), with patients generating fewer items than controls in both tasks. Patients were significantly slower in colour naming condition of the D-KEFS word-colour-interference

Table 5.4: Demographic and disease characteristics of multiple sclerosis patients and healthy controls.

	RRMS patients (n = 48)	Controls (n = 48)	Z-/t-/X^2 Score	p-value
Demographics				
Age, years (mean, SD)[a]	38.8 (9.3)	38.0 (9.6)	t = 0.398	0.691
Females/males (number)	37/11	37/11	X^2 = 0.00	1
Years of education (median, range)	13 (11-22)	14 (11-20)	Z = -0.846	0.398
Disease duration, years since diagnosis (mean, SD)	6.5 (5.4)	n/a	n/a	n/a
EDSS score (mean, SD)	2.69 (1.9)	n/a	n/a	n/a
MRI metrics				
T2-hyperintense volume, cm^3 (mean, SD)	8.82 (9.24)	n/a	n/a	n/a
Cortical thickness global, mm (mean, SD)[a]	2.33 (0.13)	2.42 (0.11)	t = -3.435	<0.01**
Third ventricle width, mm (median, range)	3.70 (1.2-12.7)	2.40 (1.2-7.4)	Z = -4.785	<0.01**

Abbreviations: BPF = Brain parenchymal fraction (= parenchymal volume / parenchymal volume + CSF volume), SD = standard deviation.
Unless otherwise noted statistical comparisons are based on Mann-Whitney U tests.
[a] Because of normal distribution in both samples statistical comparisons are based on t-tests.
* $p< 0.05$; ** $p< 0.01$

test, indicating slowed basic information processing (p <0.05). Moreover, patients showed higher levels of cognitive (p = 0.003) and physical (p <0.001) fatigue, as well as a higher level of depressive mood state (p = 0.025), relative to controls. Verbal and figural fluency performances were not significantly associated with age, disease duration, depressive symptoms, and cognitive or physical fatigue (all p >0.05). However, our measure of cognitive reserve was related to verbal fluency (r = 0.298; p = 0.02), while EDSS scores correlated with figural fluency performance (r = 0.310; p = 0.03). Therefore, these two variables were controlled for in further analyses.

MRI findings

Mean global cortical thickness was reduced in multiple sclerosis patients compared to controls (Table 5.4; p <0.001). Whole-brain vertex-wise analysis revealed multiple brain areas of significant cortical thinning in both hemispheres in patients compared with healthy controls (Figure 5.5). Cortical thinning was spread over broad regions of both hemispheres. Anatomical locations of clusters of decreased cortical thickness in the patients are summarised in the Supplemental Table 5.7 (see Section 5.2.6 for supplemental material).

Table 5.5: Neuropsychological test and questionnaire results

	RRMS patients	Healthy Controls	t-/Z-Score	p-value
Executive functions				
Verbal fluency, no. correct (median, range)	18.5 (9-34)	25 (15-45)	Z = -4.821	<0.01**
Figural fluency, no. correct (mean, SD)[a]	32.42 (8.3)	43.23 (8.6)	t = -6.266	<0.01**
Working memory, errors total (median, range)	3.0 (0-16)	2.0 (0-11)	Z = -1.611	0.107
Response inhibition, errors (median, range)[1]	1.0 (0-7)	0.0 (0-5)	Z = -1.805	0.071
Set shifting, errors (median, range)[2]	1.0 (0-9)	1.0 (0-5)	Z = -0.756	0.45
Questionnaires				
Cognitive fatigue (median, range)	5.5 (0-30)	2.0 (0-21)	Z = -3.216	<0.01**
Physical fatigue (median, range)	6.0 (0-15)	1.5 (0-32)	Z = -3.005	<0.01**
Total fatigue (median, range)	13.5 (0-55)	4.0 (0-36)	Z = -3.332	<0.01**
Depressive symptoms (median, range)	9.0 (0-37)	6.5 (0-23)	Z = -2.243	0.025*
Cognitive reserve				
MWT-B (median, range)	30.5 (23-37)	31.0 (25-36)	Z = -0.589	0.556

Abbreviations: MWT-B = Multiple-Choice Word Test-B; SD = Standard deviation
Unless otherwise noted statistical comparisons are based on Mann-Whitney U tests
[a] Because of normal distribution in both samples statistical comparisons are based on t-tests.
* $p< 0.05$; ** $p< 0.01$
[1] Here, corrected time-to-complete means the difference of the scaled scores between the interference condition and the basic color naming condition, as proposed by Delis et al. (2001)
[2] Here, corrected time-to-complete means the difference of the scaled scores between the interference condition and set shifting condition.

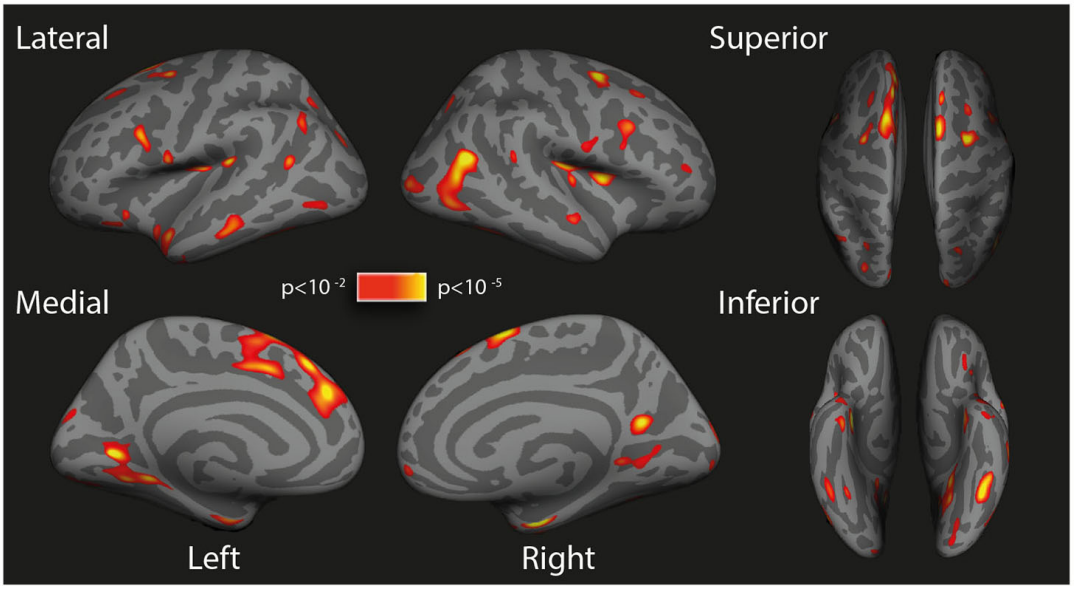

Figure 5.5: Differences in cortical thickness: Statistical maps representing significant cortical thickness differences between patients and controls on lateral, medial, inferior and superior view of inflated brains. A color scale indicates statistical significance and shows p-values. Red/yellow areas represent clusters of significant cortical thinning in patients, relative to controls.

Global analyses

The hierachical regression model accounted for 45.7% of the variance in verbal fluency performance. No predictor indicated multicollinearity (all variance inflation factors <4). After controlling for education in block one, lesion volume retained in block two, with larger lesion volume predicting worse performance in verbal fluency ($R^2 = 0.288$, $p = 0.003$). In block three, TVW as an indicator of central brain atrophy accounted for an additional 7% of variance in verbal fluency performance, with more atrophy predicting worse verbal fluency performance ($R^2 = 0.364$, $p = 0.026$). Finally, in block four global cortical thickness accounted for additional 9 % of variance in verbal fluency, with a thinner cortex being associated with worse verbal fluency performance ($R^2 = 0.457$, $p = 0.010$). In other words, cortical thickness independently predicted verbal fluency, even when controlling for lesion volume and central brain atrophy estimates. Regarding figural fluency, the full regression model accounted for 30.2% of variance in figural fluency performance. After controlling for EDSS in step one, there was a negative effect of lesion volume on figural fluency ($R^2 = 0.285$, $p = 0.009$) in block two, but no effect of brain atrophy (TVW) in block 3 ($p = 0.335$) or cortical thinning in block four ($p = 0.729$). Within the control group, neither global cortical thickness nor the TVW significantly correlated with verbal or figural fluency performance (all $p > 0.05$).

Regional analyses

Clusters of significant correlations between regional cortical thickness and verbal or figural fluency performance within the patient group are shown in Figure 5.6 and Table 5.6. Poorer performance in the verbal fluency task correlated with a thinner cortex within the left anterior cingulate, superior frontal, lateral orbitofrontal, fusiform and superior parietal region. These clusters survived FDR-correction. Performance in the figural fluency task was associated with cortical thickness in the right anterior cingulate and insula. No significant correlations were observed in the control group. Concerning the relation of T2-hyperintense lesion location with fluency, VLSM revealed no clusters of lesioned voxels associated with performance in the two fluency tasks.

5.2.5 Discussion

The results of the present study indicate that executive dysfunction in patients with multiple sclerosis can specifically affect verbal and figural fluency. For both deficits, side- and site-specific cortical correlates in the anterior cingulate region were found by means of cortical thickness analysis. Although several clusters of cortical thinning were associated with verbal and figural fluency, only one of them

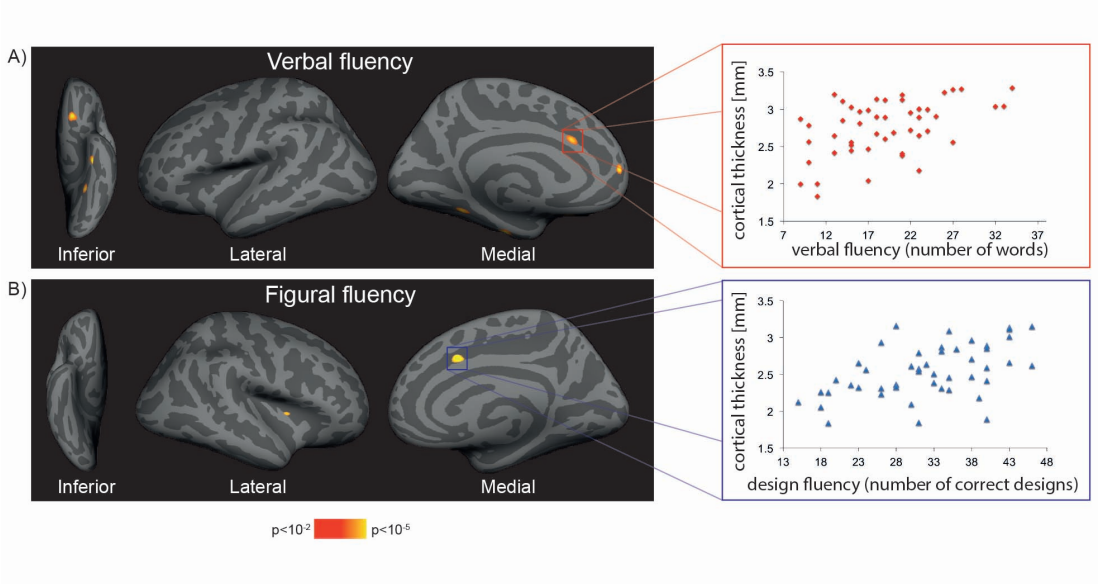

Figure 5.6: Association of cortical thickness and fluency performance: Correlation of fluency performance and cortical thickness within patients. On the left side, statistical maps show clusters of significant correlation between verbal (A) and figural (B) fluency performance and cortical thickness in the patient groups. On the right side, scatterplots illustrate the correlation between cortical thickness in the anterior cingulate cortex cluster and verbal (A), respectively fluency (B) performance.

Table 5.6: Regions of cortical thinning significantly associated with verbal and figural fluency in the patient group

Cluster Number	Talairach Coordinates x, y, z	Surface extension (mm2)	-log(p)	Structures
		Left hemisphere, medial surface		
Verbal fluency				
1	-35.5, 17.1, -19.6	109.6	5.412	Fusiform
2	-10.5, 52.3, 9.6	50.75	4.999	Superior frontal
3	-14.2, 23.8, -16.4	85.84	4.956	Lateral orbitofrontal
4	-34.0,-48.4, -10.2	58.35	4.739	Fusiform
5	-8.3, 21.8, 27.9	44.60	5.676	Caudal anterior cingulate
		Right hemisphere, medial and lateral surfaces		
Figural fluency				
1	13.1, 10.6, 37.3	46.01	5.386	Anterior cingulate
2	34.1, 3.3, 7.6	17.61	4.664	Insula

All p-values corrected for multiple comparison using FDR = 0.05. Small significant clusters are not shown (Cluster size <10mm2). Anatomical terms are used according to the Desikan-Killiany template.

was congruent across the two hemispheres, i.e. the one in the anterior cingulate cortex. The main finding of the present study is the lateralized characteristic of the correlational analyses: Thinning in the left anterior cingulate cortex predicted verbal – but not figural – fluency, while the opposite pattern was found for thinning in the right anterior cingulate cortex. This lateralisation corresponds well with lesion studies in patients with focal brain damage linking verbal fluency with the left prefrontal cortex and figural fluency with the right prefrontal cortex (Robinson, Shallice, Bozzali, & Cipolotti, 2012; Schwartz & Baldo, 2001). These studies were based on patients with relatively large focal lesions, for example due to stroke or brain tumours. On the basis of focal grey matter atrophy, our study demonstrates the same lateralised clinico-anatomical correlation pattern in MS, which is traditionally seen as a white matter disease with widespread pathology.
Previous studies showed that tests of verbal fluency are amongst the most sensitive neuropsychological instruments to assess cognitive impairment in multiple sclerosis, relative to other measures of executive functioning (Henry & Beatty, 2006). Fluency tasks in general have a long tradition in the examination of one of the most important executive function, i.e. the voluntary generation of new series of responses. Sustained activation for the duration of the task is required in order to generate non-overlearned responses for all types of fluency tasks (Robinson et al., 2012). Performance deficits on fluency tasks are due to a failure of "energisation" (Stuss & Alexander, 2007), that is, the process of initiating and sustaining any response, which is required for the generation of new responses.
In this framework, the fluency deficits found in the present study could be relabelled "cognitive hypoenergisation". It might be tempting to view this latter concept as identical to that of cognitive fatigue. However, in line with previous studies showing that self-reported fatigue is not associated with cognitive deficits in multiple sclerosis (Morrow et al., 2009), we did not find a significant correlation between fluency performance and self-reported cognitive fatigue in our patient sample.
Patients with large medial frontal lobe lesions that involve the cingulate cortex often show deficits in spontaneous initiation of speech and movement and/or an inability to suppress externally triggered subroutines (Paus, 2001). Akinetic mutism, which is caused by bilateral medial frontal lesions, represents an extreme example of this clinico-anatomical association (Barris & Schuman, 1953). Robinson and colleagues (2012) conclude that energization processes – necessary for fluency tasks – are bilaterally represented in the medial frontal region. To the best of our knowledge, we here show for the first time that the relation between anterior cingulate pathology and hypoenergetic cognitive functioning can also be found in patients with multiple sclerosis.

With regard to the clinical assessment of executive functions in multiple sclerosis, we propose that the inclusion of fluency tasks is particularly relevant. These brief tests are sensitive to executive impairment and – as suggested by our findings – cortical involvement, providing clinicians' efficient tools in situations when examination time is limited.

Comparable to previous studies, we could only find modest association between T2-hyperintense lesion burden and cognitive test performance. Roosendaal and colleagues (2009) suggest that one main reason of this is that pathology outside the focal white matter lesions, in the so-called normal appearing white matter (NAWM), remains largely undetected by conventional MRI. By now, there is evidence that advanced MRI sequences, such as diffusion tensor imaging (DTI) exhibit more robust relationship with cognitive variables (e.g. Roosendaal et al., 2009; Van Hecke et al., 2010), as it provides in-vivo information about the orientation and integrity of WM fibre bundles. Thus, NAWM changes should be investigated as an additional mechanism related to cognitive dysfunction beside T2-hyperintense lesion volume, central and cortical atrophy. Furthermore, DTI – potentially in combination with fMRI – would allow analyses of neuroconnectivity and neuronal net- works. As cognitive functions and thus also fluency performances are likely based on a network of brain regions – rather than on a single region – additional knowledge could be gained with such analyses. Based on previous and our findings, we propose that the AC is an important node of the fluency network.

The present study is not without limitations: First, only MS patients suffering from the relapsing-remitting subtype were included, so that the generalisation of the findings to other forms of multiple sclerosis such as the primary progressive variant is debatable. Furthermore, the cross-sectional design of the study does not allow causal interpretation of the observed association between structural MRI changes and cognitive impairment.

5.2.6 Supplemental material

Table 5.7: Clusters of cortical areas (left and right hemisphere) showing significant cortical thinning in relapsing-remitting MS patients compared to healthy controls

Cluster Number	Talairach Coordinates x, y, z	Surface extension (mm^2)	-log(p)	Structures
		Left hemisphere		
1	-18.3 -67.3 0.7	965.56	6.1365	lingual
2	-110.3	346.46	5.709	fusiform
3	-7.5 38.6 30.9	2019.97	-5.4904	superior frontal
4	-36.5 -36.5 16.5	130.09	5.0554	supra marginal
5	-34.2 8.6 25.2	134.52	4.5666	caudal middle frontal
6	-22.4 -4.3 46.1	125.03	4.5499	caudal middle frontal
7	-49.6 10.3 -19.1	384.85	4.3821	superior temporal
8	-40.8 -53.5 12.7	94.95	4.2618	inferior parietal
9	-33.5 -20.6 18.3	153.56	4.2511	insula
10	-60.9	125.24	4.2286	entorhinal
11	-58.0 3.7 10.1	129.66	4.1442	precentral
12	-104.5	261.83	4.0364	middle temporal
13	-42.2 -63.3 36.3	174.07	3.7251	inferior parietal
14	-28.8 -78.0 14.1	94.9	3.6089	inferior parietal
15	-26.6 24.1 -8.3	57.94	3.5256	lateral orbitofrontal
16	-65	91.34	3.5171	superior temporal
17	-24.1 -60.9 41.2	78.04	3.3917	superior parietal
18	-118.5	121.52	3.2174	lateral occipital
19	-33.6 23.6 -19.3	95.67	3.2063	lateral orbitofrontal
20	-13.6 -16.6 44.2	35.23	3.1067	paracentral
21	-117.1	200.44	3.0842	lateral occipital
22	-21.5 20.5 41.8	78.75	3.073	superior frontal
23	-5.2 -83.9 26.1	124.36	2.9508	cuneus
24	-84.1	81.36	2.9237	inferior temporal
25	-5.5 -60.2 14.4	35.37	2.8229	precuneus
26	-43.8 -68.3 7.9	16.07	2.6849	inferior parietal
		Right hemisphere		
1	24.9 -6.5 -31.	101.61	6.6866	entorhinal
2	41.3 -57.9 17.6	897.57	5.847	inferior parietal
3	9.5 8.4 65.1	366.74	5.7239	superior frontal
4	24.4 -3.8 45.5	267.35	5.5027	precentral
5	12.9 -56.0 18.9	203.81	5.2272	precuneus
6	33.6 2.6 13.3	182.28	4.9933	insula
7	34.6 -21.7 20.7	303.28	4.6877	insula
8	10.6 27.8 55.3	92.63	4.2329	superior frontal
9	35.4 -5.7 -35.9	133.16	4.1083	fusiform
10	32.5 11.3 30.2	179.98	4.0422	caudal middle frontal
11	28.4 -88.5 1.2	261.28	3.905	lateral occipital
12	19.5 -45.8 -2.0	314.41	3.7091	isthmus cingulate
13	42.1 -51.5 -11.6	119.44	3.6543	fusiform
14	49.9 -10.6 -13.9	114.26	3.6519	superior temporal
15	32.8 -53.9 -15.8	55.73	3.6105	fusiform
16	11.1 -91.7 13.7	177.02	3.5916	cuneus
17	30.1 17.5 44.8	95.06	3.5655	caudal middle frontal
18	20.9 -62.0 41.1	83.6	3.5186	superior parietal
19	10.8 55.8 -5.4	82.28	3.4249	medial orbitofrontal
20	33.5 14.9 -36.3	123.59	3.3771	temporal pole
21	60.6 -41.3 15.5	60.55	3.3744	bankssts
22	15.3 -93.9 -6.0	74.21	3.2249	pericalcarine
23	11.5 -4.6 46.9	45.83	3.2019	superior frontal
24	54.1 -38.6 45.2	35.43	3.1808	supramarginal
25	60.5 -5.0 13.4	120.15	3.1535	postcentral
26	52.2 -4.8 9.9	35.23	2.9518	precentral
27	37.9 42.6 10.3	60.32	2.9027	rostral middle frontal
28	47.9 -34.3 12.4	45.6	2.8328	superior temporal
29	59.0 -56.2 4.8	37.85	2.7796	middle temporal

Anatomical terms are used according to the Desikan-Kiliany template.

5.3 Study 3

Random number generation deficits in patients with Multiple Sclerosis: Characteristics and neural correlates

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Under review in
Cortex

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5.3.1 Abstract

Human subjects typically deviate systematically from randomness when attempting to produce a sequence of random numbers. Despite an increasing number of behavioral and functional neuroimaging studies on random number generation (RNG), its neuroanatomical underpinnings have never been investigated. We set out to fill this gap in 44 patients with multiple sclerosis (MS), a disease whose impact on RNG has never been studied. The RNG task required the paced (1 Hz) generation of the numbers from 1 to 6 in a sequence as random as possible. The same task was administered in 39 matched healthy controls. To assess neuroanatomical correlates such as cortical thickness, lesion load and third ventricle width, all subjects underwent high-resolution structural MRI. Compared to controls, MS patients exhibited an enhanced tendency to arrange consecutive numbers in an ascending order ("forward counting"). Furthermore, patients showed a higher susceptibility to rule breaks (producing out-of-category digits like 7) and to skip beats of the metronome. Clinico-anatomical correlation analyses revealed two main findings: First, increased counting in MS patients was associated with higher cortical lesion load. Second, increased number of skipped beats was related to widespread cortical thinning. In conclusion, our test results illustrate a loss of behavioral complexity in the course of multiple sclerosis, while the imaging results suggest an association between this loss and cortical pathology.

5.3.2 Introduction

As smart human brains are in spotting patterns and following rules, as miserably they fail in attempting to be unpredictable. A case in point is our inability to generate random sequences of responses. Instructions of a random number generation (RNG) task require subjects to arrange numbers in a sequence "as random as possible", implicitly asking to avoid any algorithm and to disobey any rule. Under a huge range of conditions (Brugger, 1997) healthy volunteers were found unable to follow these instructions, and so were patients with various neuropsychiatric diseases (Brown, Soliveri, & Jahanshahi, 1998; Brugger et al., 1996; Ho, Sahakian, Robbins, & Barker, 2004; Salamé & Danion, 2007; Spatt & Goldenberg, 1993). A limited capacity of working memory and executive functions have been implied in the failure to produce unpredictable, or random sequences of response alternatives (Baddeley, 1966; Baddeley et al., 1998; Joppich et al., 2004; Maes, Eling, Reelick, & Kessels, 2011; Miyake et al., 2000). Neuroimaging studies have largely supported the assumed implication of the (pre)frontal lobes for RNG (Artiges et al., 2000; Itagaki, Niwa, Itoh, & Momose, 1995) and non-invasive intervention methods have suggested their causal involvement (Jahanshahi et al., 1998; Knoch, Brugger, & Regard, 2005).

Facing the large and rapidly growing number of behavioral and functional neuroimaging studies on RNG, one is left wondering why the neuroanatomical correlates of RNG have never been examined. The present study was aimed at filling this gap in patients with multiple sclerosis (MS), a disease whose impact on RNG has, to the best of our knowledge, never been studied. MS, the most common autoimmune disorder affecting the central nervous system, is historically considered a white matter (WM) disease, with focal demyelinating lesions in the WM being the pathological hallmark. However, several recent neuropathological studies disclosed a relevant involvement of gray matter areas including the cerebral cortex (M. Calabrese et al., 2012; Geisseler et al., 2016). Brain integrity, captured by various imaging techniques, has been shown to correlate with cognitive impairment (for review see (for review see Rocca et al., 2015), which is recognized as an important feature of MS, prevalent in 43-70% of MS patients (Chiaravalloti & DeLuca, 2008). Although processing speed (e.g. DeLuca et al., 2004; Roth et al., 2015) and episodic memory (e.g. Rogers & Panegyres, 2007) seem to be the most prominent cognitive feature of MS, these patients often exhibit significant deficits in executive functions (Geisseler et al., 2016; Henry & Beatty, 2006). Against this background, it appears tempting to assume that MS patients exhibit impaired performance in generating random sequences of numbers.

The aim of the present study is two-fold. On the one hand, we planned to examine

the impact of MS on randomization performance; on the other hand, we wanted to explore, for the first time, the neuroanatomical correlates of RNG. Specifically, we predicted an impaired randomization performance by the patients with MS relative to a carefully matched healthy control group. As a first step in uncovering the neuroanatomical correlates of RNG, we planned to analyze associations between brain structure and RNG performance in healthy participants and, in the patient group, between cortical and subcortical damage and RNG performance.

5.3.3 Methods

Participants

Forty-four patients with a definite diagnosis of relapsing-remitting multiple sclerosis (RRMS) according to the 2010 McDonald criteria (Polman et al., 2011) and 39 age-, gender-, handedness- and education-matched healthy controls participated in this study. Inclusion criteria for the patient group were no relapse or steroid-treatment during the last two months, no current or past neurological disorder in addition to multiple sclerosis, and no psychiatric disorders apart from MS-related depressive mood state. The local ethics committee approved the study, and all subjects gave written informed consent before participation. Control participants received financial compensation.

RNG task, performance measures and hypotheses

The Mental Dice Task (MDT) was administered in its standardized form (Brugger et al., 1996). Subjects were instructed to imagine repeatedly throwing a die and to orally report the number that would show up, i.e., they had to generate numbers from 1 to 6 in a random fashion. Subjects were instructed to synchronize their response with a pacing auditory stimulus, which was a beeping sound presented at 1 Hz. A total of 66 valid responses were recorded.
While there are many different measures of (non)randomness, we focused on three variables, which were calculated by means of a freely available computer program (http://www.lancs.uk/staff/towse/rgcpage.html). First, we determined the distribution of first-order differences (FODs), reflecting the arithmetic difference between each response and the preceding one. Thus, FODs may vary between -5 (6 followed by 1) and +5 (1 followed by 6). A value of 0 is scored when any of the six numbers are repeated. The frequency of zero values can be used to measure repetition avoidance, with fewer zeroes indicating more alternations. We predicted a comparable degrees of repetition avoidance for both groups, as repetition (alternation) behavior may be under hippocampal, rather than prefrontal

control (Lalonde, 2002). An FOD value of +1 reflects a counting strategy, which we predicted to be pronounced in patients with MS, under the assumption of an impaired executive control of automatized responding. Figure 1 displays all possible pair combinations and the expected frequencies of the resulting 11 different FODs. Second, the frequency of shifts between ascending and descending sequences is captured by the turning point index (TPI). TPI is reported as a percentage score, meaning that values greater than 100 indicate too many turning points - relative to a theoretical distribution of random responses - whereas values less than 100 indicate fewer turning points than expected. We predicted that patients showed a lower TPI than patients, again because an impaired suppression of automatized counting upwards and downwards would lead to longer ascending and descending sequences. Finally, as a global measure of randomness, Evans' random number generation index (Evans (1978); RNG index) was calculated, ranging from 0 to 1, with higher indices representing less randomness, which was predicted for the patient group. Further information and mathematical details of these calculations are described elsewhere (Towse & Neil, 1998).
Additionally, and besides these measures of sequential non-randomness, two non-sequential variables of the MDT were considered, i.e., the number of rule breaks (i.e., producing out-of-category digits such as 7) and the total number of skipped beats of the metronome.

MRI data acquisition, post-processing and hypotheses

The MRI scan was performed within one month of the neuropsychological examination. All images were acquired using a 1.5-T scanner (Siemens Magnetom AvantoTM) equipped with a SQ-engine gradient (45m/T/m @ 200 T/m/s) using a dedicated 32-channel head coil. No hardware upgrades of the scanner occurred during the study period. The following sequences were obtained from all subjects: (1) Double Inversion Recovery sequence (DIR) (voxel size =1.5 x 1.5 x 1.5 mm, slice thickness = 1.5 mm, repetition time = 7500ms, echo time = 308ms); (2) T1-weighted MPRAGE (voxel size = 1 x 1 x 1 mm, slice thickness = 1 mm, repetition time = 2420 ms, echo time = 4.18 ms) and (3) a T2-weighted FLAIR (voxel size = 0.9 x 0.9 x 2.0 mm, slice thickness = 2 mm, repetition time = 5000 ms, echo time = 342 ms). Cortical lesions were defined according to the consensus recommendations of Geurts and colleagues (2011). Consequently, cortical lesions are those lesions appearing hyperintense on DIR images compared to surrounding normal-appearing grey matter, entirely or partly located in the cortical grey matter and occupying at least three voxels. DIR-hyperintense lesions were identified and manually delineated with MRIcron (http://sph.sc.edu/comd/rorden/mricron), which was further used to measure total lesion volume. The same procedure was applied to FLAIR

Table 5.8: Demographic characteristics and measures of performance on the MDT of MS patients and healthy controls

	MS patients	Controls	*p*-value
Number	44	39	-
Gender [m/f]	10/34	6/33	0.397
Age [years], mean (SD)	39.02 (9.4)	38.53	0.813
Education [years], mean (SD)	14.6 (3.1)	14.3(2.4)	0.69
EDSS, mean	2.6	-	-
Disease duration [months], mean	79.5	-	-
RNG, mean	0.47	0.43	0.151
Counting [FOD 1], mean	15.91	11.54	0.004**
Repetition avoidance [FOD 0], mean	3.11	3.51	0.699
TPI, mean	77.02	89.84	< 0.001***
Skipped beats, mean	6.52	0.18	< 0.001***
Rule breaks, mean	0.37	0.1	0.014*

Abbreviations: EDSS = Expended disability status scale, RNG = Random number generation, FOD = First order difference, TPI = Turning point index.

images to identify and segment T2-hyperintense lesions. An experienced rater assessed all images, supervised by a neuroradiologist. Central brain atrophy was examined by measuring the width of the third ventricle, implemented according to the procedure defined by Benedict and colleagues (2006). Similar to many other studies on cognition in MS (e.g. Benedict, Bruce, et al., 2006), we expect a wider third ventricle to be associated with poorer behavioral performance. Cortical thickness evaluation was performed by means of Freesurfer image analysis suite, which is documented and freely available online (http://surfer.nmr.mgh.harvard.edu). Further information and technical details of these procedures are described in prior publications (Dale et al., 1999; Fischl et al., 1999). To detect possible misclassifications of white and grey matter due to multiple sclerosis lesions, all images were visually inspected after the segmentation of grey and white matter. In one patient, a semi-automated correction of topological defects was required by manually adding control points. Global cortical thickness as well as cortical thickness estimates for the regions resulting from automated parcellations (Desikan et al., 2006) were extracted from the Freesurfer software for further analyses. In line with the hypothesis that RNG performance rely on frontal functions, we mainly focused on frontal regions of interest (ROI), whereas temporal and parieto-occipital ROI were used as control regions.

Statistical analyses

Statistical analyses of the demographical and cognitive data, including group comparisons, were performed with SPSS (IBM, Chicago, USA, Version, 21.0,

http://spss.com). Unless otherwise stated, a p-level below 5% was considered statistically significant. Assumptions for normality were tested for all continuous data with Kolmogorov-Smirnov tests. In case of normally distributed variables, group comparisons and correlational analyses were based on parametric tests (independent t-tests, Pearson correlation). When variables were not normally distributed, non-parametric tests (Mann-Whitney U test, Spearman correlation) were applied. Concerning all group comparisons, correlational analyses and ROI analyses, Bonferroni correction was used to prevent alpha error inflation.

5.3.4 Results

RNG findings

Demographic characteristics and MDT test results are reported in Table 5.8. The distribution of FODs is shown in Figure 5.7, and an overview of the RNG parameters is given in Table 5.8. Patients displayed significantly ($T(67.9) = 3.010$; $p = 0.004$) more forward counting steps (Mean = 15.91; SD = 8.335) than healthy controls (Mean = 11.54; SD = 4.541). Furthermore, the latter showed more backward counting steps of two than the patients (FOD = -2; $T(81) = -2.490$; $p = 0.015$). No differences between groups were observed in the number of repetitions (FOD = 0). Patients (Mean = 77.02; SD = 18.17) had a smaller TPI than controls (Mean = 89.8, SD = 13.33), i.e. they showed less changes from ascending to descending sequences and vice versa ($T(81) = -3.624$, $p < 0.001$). RNG indices did not differ significantly between groups ($Z = -1.437$; $p = 0.151$). Regarding non-sequential measures, MS patients showed a higher susceptibility to rule breaks ($Z = -2.431$; $p = 0.014$) as well as to skip beats of the metronome ($Z = -4.983$, $p < 0.001$) than the control group.
Correlational analyses within the patient group yielded no relationship between RNG parameters and age, education, disease severity (EDSS), fatigue or depressive mood (all $p > 0.050$).

MRI findings

Relative to controls, patients showed significant cortical thinning and widening of the third ventricle (both $p < 0.010$). Results of the univariate correlation analyses between the global atrophy and the RNG parameters as well as between the lesion and the RNG parameters within the patient group are summarized in Table 5.9.
Within the patient group, mean global cortical thickness showed a significant negative correlation with skipped beats in the MDT ($r_s = -0.411$, $p = 0.006$), i.e. a thinner cortex was associated with more skipped beats. In contrast, global cortical thickness was not associated with TPI, counting tendency, or the number of

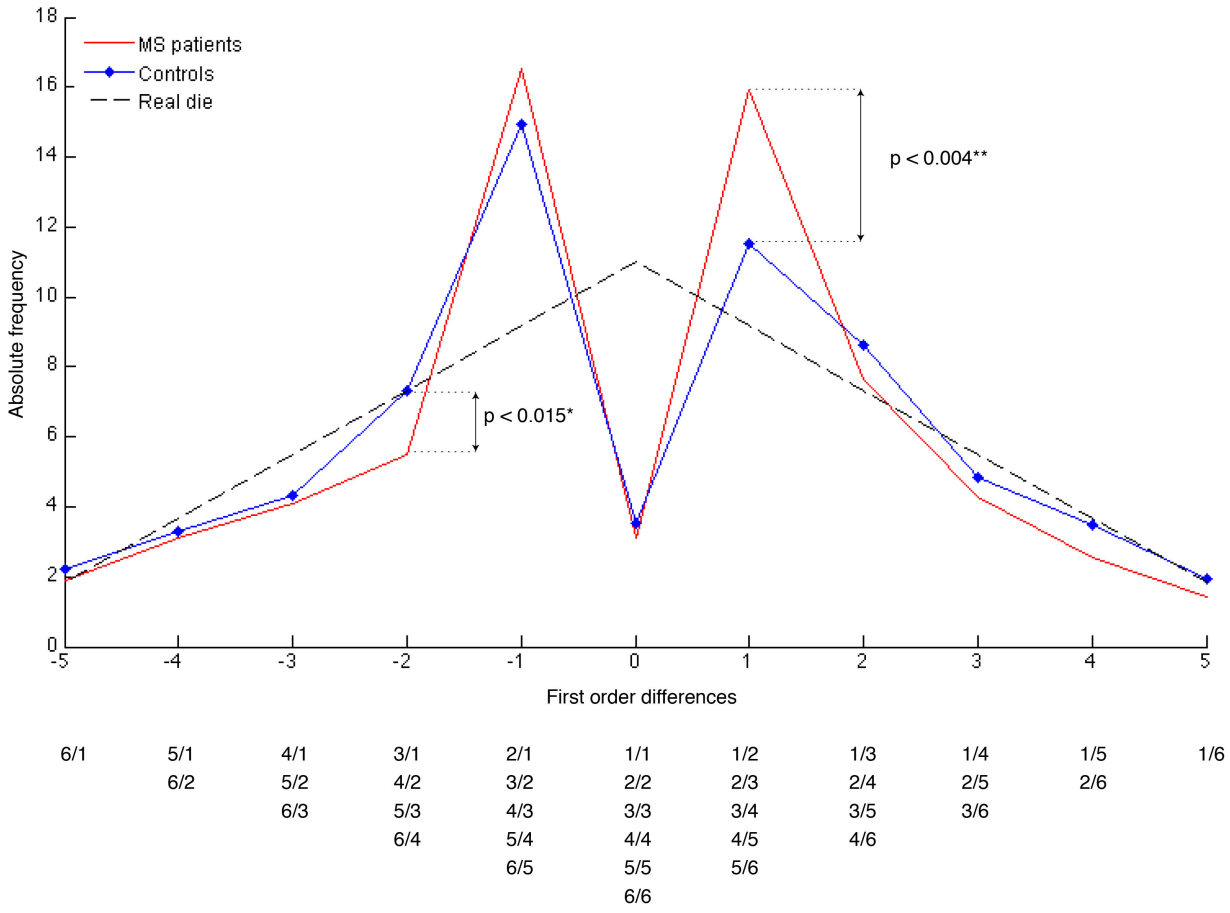

Figure 5.7: Distribution of first-order differences (FODs). Avoidance of repetitions is demonstrated at point 0 on the x-axis, forward counting at point 1, backward counting at point -1.
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Table 5.9: Spearman correlations between measures of the Mental Dice Task (MDT) and neuroimaging parameters in patients with relapsing-remitting multiple sclerosis (n=44)

	Sequential measures		Non-sequential measures	
	TPI	Counting tendency	Skipped beats	Rule breaks
	r_s(*p-value*)	r_s(*p-value*)	r_s(*p-value*)	r_s(*p-value*)
Atrophy parameters				
Cortical thickness	0.078 (*0.614*)	-0.161 (*0.296*)	-0.411(*0.006***[a])	0.112 (*0.467*)
Third ventricle width	-0.110 (*0.477*)	0.332 (*0.028*)	0.237 (*0.122*)	0.174 (*0.259*)
Lesion parameters				
T2-hyperintense lesion load	-0.190 (*0.217*)	0.270 (*0.076*)	0.345 (*0.022*)	0.015 (*0.922*)
Cortical lesion load	-0.053 (*0.731*)	0.402 (*0.007***[a])	0.306 (*0.043*)	-0.094 (*0.545*)

Abbreviations: TPI = Turning point index
[a] The correlation remained significant when using a stringent Bonferroni correction (alpha = 0.05/8 = 0.00625)
* $p < 0.05$; ** $p < 0.01$

rule breaks (all $p > 0.050$). The width of the third ventricle did not correlated with any of the RNG parameters. Regarding lesion parameters, DIR-hyperintense lesion volume correlated with the counting tendency ($r = 0.402$; $p < 0.007$; Figure 5.8). However, both DIR-hyperintense cortex-involving ($r = 0.306$; $p = 0.045$) as well as FLAIR-hyperintense lesion volumes ($r = 0.345$; $p = 0.022$) were uncorrelated with the TPI and the non-sequential parameters. None of the global MRI variables correlated with TPI or the number of rule breaks.

On a regional level, ROI analyses revealed no significant correlation between counting tendency and the cortical thickness in frontal regions of interest when applying strict Bonferroni correction. Supplementary Table 5.10 (supplemental material in Section 5.3.6) shows r- and uncorrected p-values (which ranged between $r = -0.359$, $p < 0.009$ and $r = 0.018$, $p < 0.454$). Similarly, no significant correlation was observed between the temporal and parietal control ROIs with any sequential parameters. Regarding non-sequential parameters, the ROI-based correlational analyses did also not survive Bonferroni correction; Supplementary Table 5.11 (in Section 5.3.6) displays uncorrected p-values.

Within the control group, the only significant correlation between global cortical thickness and the RNG parameters was that with skipped beats ($r_s = -0.342$, $p < 0.033$). No significant correlations were observed between the width of the third ventricle or global cortical thickness and any of the RNG parameters, and the regional ROI analyses were also uncorrelated with both sequential and non-sequential measures of the MDT (see Supplementary Table 5.12 for uncorrected correlation values).

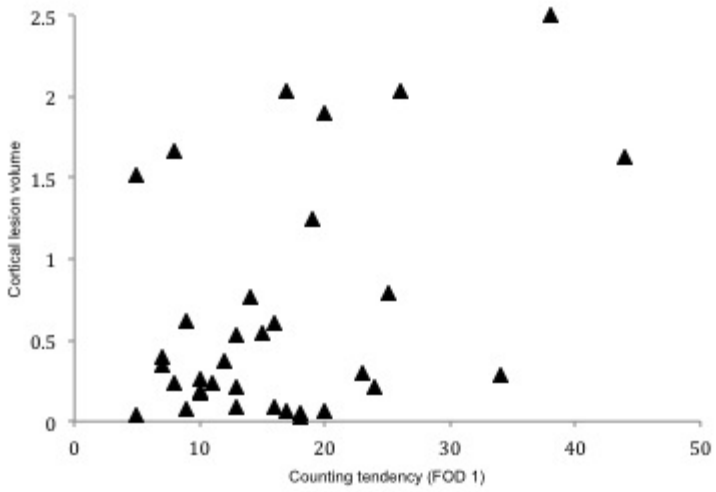

Figure 5.8: Correlation between cortical lesion volume (cm^3) and the counting tendency (frequency of FOD 1)

5.3.5 Discussion

The current study examined the behavioral performance and neuroanatomical underpinnings of RNG in a cohort of MS patients, compared with a group of matched healthy controls. As predicted, random number sequences generated by MS patients were more stereotyped than those of healthy controls. More precisely, MS patients showed more ascending counting behavior than control subjects. This corresponds well with previous reports of a deterioration of randomization performance in the presence of a neurological disorder (Brown et al., 1998; Brugger et al., 1996; Ho et al., 2004) and with the observation of executive dysfunction in MS (Drew et al., 2008). Patients did not differ from controls in the global measure of randomness (RNG index; 0.47 and 0.43, respectively), perhaps due to a "floor effect"; both groups scored far from the value obtained in 100 simulations of 66 dice throws, i.e. 0.36 (see Brugger et al. (1996)). Relative to healthy controls, patients also exhibited problems in non-sequential parameters. They showed pronounce difficulties in keeping pace (1Hz) and in avoiding invalid numbers (e.g. 7). The former problem is consistent with reports that the primary cognitive deficit in MS patients may be generalized slowing of information processing speed (DeLuca et al., 2004). Thus, compared with healthy controls, MS patients completed substantially fewer items on rapid serial processing tests such as for example the Paced Auditory Serial Addition Test (PASAT; Parmenter, Shucard, and Shucard (2007)). Consequently, the non-sequential variable of skipped beats in the RNG may be a useful parameter for a brief and valid assessment of information processing speed. Cognitive impairment in MS patients has been related to a number of structural abnormalities in the past. Demyelinated T2 lesions in the white matter – the

pathological hallmark of MS – are only modestly correlated with cognitive impairment (Lazeron et al., 2005; Rao et al., 1989). Higher correlations are observed between cognitive performance and third ventricle width, which is considered the best single MRI predictor for MS-related cognitive impairment
(Benedict, Bruce, et al., 2006; Benedict et al., 2004). More recent research highlights cortical pathology as one of the major substrates of cognitive decline in MS (for review see Messina & Patti, 2014). Our clinico-anatomical correlation analyses revealed two main findings that survived Bonferroni correction: First, increased counting in MS patients was associated with higher cortical lesion load. Second, increased number of skipped beats were related to widespread cortical thinning. No structural correlates could be found for the number of rule breaks and the TPI-measure of sequential randomness. FLAIR-hyperintense lesion load did not correlate significantly with any of the behavioral parameters.
The association between a widespread thinner cortex and an increased number of skipped beats is among the most robust findings of the present study. Previous studies showed that the volume or thickness of the neocortex is correlated with mental processing efficiency in MS (Amato et al., 2007; Benedict, Bruce, et al., 2006). We propose that skipped beats tightly reflect speed and attention, both in MS patients and healthy controls.
To the best of our knowledge, this is the first study evaluating the RNG performance by means of structural MRI. Our results suggest an association between cortical pathology and RNG performance. On a behavioral level, it can be concluded that even when compensating for difficulties by slowing the rate at which they produced numbers, MS patients commit rule breaks and cannot sufficiently suppress a counting algorithm. RNG may be a useful tool in the clinical monitoring of MS. As the task is highly resistant to practice effects (Brugger, 1997; Jahanshahi, Saleem, Ho, Dirnberger, & Fuller, 2006), it may prove particularly suitable for monitoring cognitive capacity over time.
Future studies could employ lesion-symptom mapping analyses in patients with focal lesions to establish connections between brain circuitry and selected measures of sequential non-randomness. Structural connectivity analyses will also be needed to better understand neurostructural underpinnings of randomization performance in health and disease.

5.3.6 Supplemental material

Table 5.10: Spearman correlation of sequential parameters with the cortical thickness of ROIs

	Left hemisphere				Right hemisphere			
	TPI		FOD1		TPI		FOD1	
	r_s	*p-value*	r_s	*p-value*	r_s	*p-value*	r_s	*p-value*
Frontal								
caudal middle frontal	0.149	0.168	-0.051	0.371	-0.038	0.402	0.103	0.253
lateral orbito frontal	0.067	0.332	-0.181	0.12	-0.044	0.389	-0.016	0.459
medial orbito frontal	-0.105	0.249	-0.061	0.348	-0.169	0.137	-0.03	0.422
pars opercularis	-0.012	0.468	0.100	0.259	-0.08	0.304	0.047	0.380
pars orbitalis	0.115	0.229	-,358	0.009	0.052	0.369	-0.147	0.171
pars triangularis	0.184	0.116	-,294	0.027	0.192	0.106	-,321	0.017
precentral	0.11	0.239	-0.024	0.439	0.163	0.146	-0.097	0.266
rostralmiddlefrontal	0.11	0.238	-0.177	0.125	0.121	0.217	-0.203	0.093
superior frontal	0.16	0.15	-0.101	0.257	0.149	0.168	-0.064	0.339
frontal pole	-0.049	0.375	0.018	0.454	0.122	0.215	-0.17	0.135
Temporal								
superior temporal	0.225	0.071	-0.18	0.121	0.078	0.307	-0.114	0.23
middle temporal	0.127	0.205	-0.049	0.377	0.051	0.37	-0.071	0.324
inferior temporal	0.167	0.139	-0.069	0.329	0.065	0.338	0.022	0.443
fusiform	0.042	0.393	-0.02	0.448	0.062	0.345	-0.011	0.472
transverse temporal	-0.13	0.2	0.15	0.166	0.062	0.346	0.047	0.38
entorhinal	0.062	0.345	-0.047	0.382	-0.067	0.333	0.043	0.392
temporale pole	0.096	0.269	-0.093	0.274	0.083	0.295	-0.166	0.141
parahippocampal	0.207	0.089	-0.131	0.198	0.087	0.288	-0.044	0.39
Parieto-occipital								
superior parietal	0.017	0.456	-0.006	0.484	0.125	0.21	-0.014	0.464
inferior parietal	0.116	0.227	-0.059	0.351	0.028	0.429	0.051	0.372
supramarginal	0.064	0.341	-0.095	0.27	0.004	0.49	-0.043	0.391
postcentral	0.122	0.216	-0.079	0.306	-0.116	0.227	0.115	0.228
precuneus	0.166	0.141	-0.129	0.203	0.052	0.368	-0.077	0.309
lateral occipital	0.027	0.43	0.04	0.397	0.094	0.272	-0.092	0.277
lingual	-0.085	0.292	0.102	0.256	-0.061	0.348	0.031	0.422
cuenus	-0.099	0.262	0.074	0.317	0.113	0.232	-0.138	0.186
pericalcarine	-0.182	0.119	0.201	0.096	0.19	0.109	-0.153	0.16

Table 5.11: Spearman correlation of nonsequential parameters with the cortical thickness of ROIs

	Left hemisphere				Right hemisphere			
	Skipped beats		Rule breaks		Skipped beats		Rule breaks	
	r	*p-value*	*r*	*p-value*	*r*	*p-value*	*r*	*p-value*
Frontal								
caudal middle frontal	-,370	0.007	-0.022	0.443	-0.251	0.05	0.207	0.088
lateral orbito frontal	-,294	0.027	-,314	0.019	-0.111	0.236	-0.14	0.183
medial orbito frontal	-0.245	0.055	-0.131	0.198	-,288	0.029	0.106	0.246
pars opercularis	-0.191	0.107	0.121	0.217	-0.244	0.055	-0.001	0.496
pars orbitalis	-0.125	0.21	-0.089	0.284	-0.119	0.221	0.135	0.192
pars triangularis	-,312	0.02	-0.104	0.25	-0.23	0.066	0.03	0.422
precentral	-,415	0.003	0.013	0.467	-,311	0.02	0.096	0.267
rostralmiddlefrontal	-,388	0.005	0.029	0.427	-,286	0.03	0.068	0.331
superior frontal	-,381	0.005	0.071	0.323	-,401	0.004	0.05	0.373
frontal pole	-0.091	0.278	0.249	0.051	-,293	0.027	0.238	0.06
Temporal								
superior temporal	-,413	0.003	-0.147	0.17	-0.195	0.102	0.037	0.407
middle temporal	-,378	0.006	0.242	0.057	-0.207	0.089	,338	0.012
inferior temporal	-0.224	0.072	0.111	0.236	-,284	0.031	0.224	0.072
fusiform	-,389	0.005	0.104	0.25	-,396	0.004	0.155	0.158
transverse temporal	-0.11	0.238	0.142	0.179	-0.15	0.165	0.071	0.324
Entorhinal	-,294	0.026	0.19	0.108	-,268	0.04	0.194	0.104
Temporal pole	-,422	0.002	0.076	0.313	-,403	0.003	-0.063	0.343
parahippocampal	-0.128	0.203	-0.094	0.272	-0.213	0.082	-0.166	0.141
Parieto-occipital								
superior parietal	-0.249	0.052	,260	0.044	-,269	0.039	0.234	0.063
inferior parietal	-,367	0.007	0.223	0.073	-,260	0.044	,308	0.021
supramarginal	-,251	0.05	0.181	0.12	-0.173	0.13	,287	0.029
postcentral	-,267	0.04	0.167	0.14	-0.227	0.069	0.186	0.113
precuneus	-,291	0.028	0.108	0.243	-,298	0.025	0.064	0.339
lateral occipital	-,361	0.008	,305	0.022	-,356	0.009	,301	0.024
lingual	-,303	0.023	0.156	0.157	-,436	0.002	0.022	0.444
cuenus	-0.162	0.147	0.179	0.122	-0.187	0.112	,366	0.007
pericalcarine	-0.016	0.459	0.072	0.322	-0.139	0.185	-0.078	0.307

Table 5.12: Spearman correlation of nonsequential parameters with the cortical thickness of ROIs in the control group

	Left hemisphere				Right hemisphere			
	Skipped beats		Rule breaks		Skipped beats		Rule breaks	
	r	*p-value*	r	*p-value*	r	*p-value*	r	*p-value*
Frontal								
caudal middle frontal	-.320	0.023	-0.124	0.226	-0.261	0.054	-0.128	0.219
lateral orbito frontal	-0.23	0.079	0.038	0.41	-0.036	0.415	-0.124	0.226
medial orbito frontal	-0.136	0.204	0.023	0.446	0.012	0.47	-0.053	0.375
pars opercularis	-.303	0.03	-0.113	0.247	-.296	0.034	-0.135	0.206
pars orbitalis	0.175	0.143	0.21	0.099	-0.019	0.454	-.300	0.032
pars triangularis	-.271	0.048	-0.139	0.199	-0.198	0.113	-0.083	0.309
precentral	-.268	0.049	-0.21	0.099	-0.25	0.062	-0.173	0.147
rostralmiddlefrontal	-.341	0.017	-0.045	0.393	-0.189	0.125	-.270	0.048
superior frontal	-.318	0.024	-0.158	0.169	-.347	0.015	-0.229	0.08
frontal pole	-0.261	0.054	-0.038	0.41	-0.187	0.127	-0.143	0.193
temporal								
superior temporal	-.351	0.014	-0.12	0.233	-.338	0.018	-0.195	0.117
middle temporal	-.407	0.005	-0.165	0.157	-0.23	0.08	-0.143	0.193
inferior temporal	-0.257	0.057	-0.165	0.157	-0.25	0.063	-0.15	0.181
fusiform	-0.232	0.078	-0.165	0.157	-0.123	0.228	-0.008	0.482
transverse temporal	-.290	0.037	-0.045	0.393	-.423	0.004	-.270	0.048
Entorhinal	0.038	0.41	-0.143	0.193	-0.01	0.476	-0.21	0.099
Temporal pole	-0.092	0.289	0.098	0.277	0.068	0.34	0.161	0.163
parahippocampal	-0.181	0.135	-0.075	0.325	0.021	0.449	-0.075	0.325
Parieto-occipital								
superior parietal	-0.208	0.101	0.094	0.285	-0.223	0.086	-0.101	0.270
inferior parietal	-0.252	0.061	-0.135	0.206	-0.193	0.119	-0.06	0.358
supramarginal	-.292	0.036	-0.143	0.193	-.427	0.003	-0.218	0.091
postcentral	-.337	0.018	-0.098	0.277	-.480	0.001	-0.12	0.233
precuneus	-.359	0.012	-0.233	0.077	-.342	0.017	-.270	0.048
lateral occipital	-0.138	0.201	0.218	0.091	-0.149	0.182	0.158	0.169
lingual	-.403	0.005	-0.225	0.084	-.324	0.022	-0.105	0.262
cuenus	-0.221	0.088	-0.023	0.446	-0.077	0.321	-0.12	0.233
pericalcarine	0.058	0.364	-0.15	0.181	-0.167	0.155	-0.03	0.428

6 General Discussion

The present dissertation aimed to contribute to a better understanding of cognitive impairment in MS patients and to characterize the link between neurostructural changes and specific cognitive deficits. In the last part of this thesis, I will summary and discuss the findings yielded by the empirical studies by elaborating on the open research questions (see Chapter 3). Subsequently, the strengths and limitations of these studies are discussed and, finally, clinical implication and suggestions for future investigations are presented.

6.1 Cortical lesions and cognitive impairment

In the last decade several studies reported a correlation of the number and volume of cortical lesions with measures of cognitive impairment (M. Calabrese et al., 2009; Roosendaal et al., 2009), physical disability (e.g M. Calabrese et al., 2007) and other neurological symptoms such as epilepsy (M. Calabrese et al., 2011). Moreover, prognostic validity has been proposed. It has also been shown that not all MS patients show cortical lesions. However, to the best of our knowledge, no research has yet been carried out to determine differences between patients with and without cortical lesions. This fact lead to the question, whether patients with and without cortical lesions differ, on the one hand, regarding cognitive functioning, and, on the other hand, regarding other important variables such as disease duration, age or EDSS (first research question; Chapter 3). Additionally, neuroanatomical differences were brought into focus. Consequently, with our first study we sought to identify these differences. We hypothesized that patients with cortical lesions, compared to those without, would show diverse disease-associated cognitive impairment and neuroanatomical characteristics.

The findings of the first study provide support for these hypotheses. First, regarding cognitive functioning, we demonstrated that patients with and those without visible cortical lesions differ from each other in mnestic functions, whereas the groups did not deviate in any non-mnestic cognitive function. Moreover, patients without cortical lesions show normal mnestic capacity compared with healthy controls. This finding is in good agreement with previous studies stating the important role of cortical lesions on cognition (e.g. M. Calabrese et al., 2009). Interestingly,

we found the highest prevalence for cortical lesions bilateral in mesiotemporal regions, with a particular prevalence in the parahippocampal gyri - a region highly associated with memory functions. Roosendaal et al. (2009) described a comparable regional association between accumulation of cortical lesions in mesio-temporal areas and impairments in episodic memory.

Second, no differences were observed between the patient groups regarding the demographical and disease-related characteristics. In accordance with the findings of Calabrese and colleagues (2010), no significant correlation between cortical lesion and disease duration or EDSS score was revealed. However, other authors found a weak to moderate correlation between these variables (e.g Dalton et al., 2004). Our findings indicate that cortical lesions can be found independent of age, age at diagnosis or disease duration.

Third, concerning neuroanatomical differences, patients with cortical lesions exhibited a thinner cortex than the other patient group, whose cortex was unimpaired. Our findings of different cortical thickness in the two patient groups are suggestive of a relationship between cortical inflammation and cortical atrophy. Given that we do not have direct histopathological confirmation, we can only speculate that cortical inflammation, indicated by cortical lesions, contributes at least partially to the development of cortical atrophy in MS patients. So far it is known that GM pathology involves both inflammatory and degenerative mechanisms, but the relationship between the two is unclear and the question whether cortical atrophy can occur as a result of focal cortical demyelination unanswered (M. Calabrese, Magliozzi, et al., 2015). GM atrophy might be the final step of several pathological processes, which could include cortical demyelination but also retrograde degeneration secondarily to WM lesions, and perhaps, primary neurodegeneration (Geurts et al., 2012).

To conclude, in this study we show that mnestic impairment is a typical characteristic of cortical affection in MS and propose a so-called 'cortically dominant' MS subtype. Moreover, the study highlights the clinical relevance of visible DIR-hyperintense cortical lesions in patients with RRMS insofar as it shows the association with neurodegenerative cortical thinning and mnestic dysfunction. Hence, the first research question can be answered in the affirmative (see Chapter 3) in terms of showing differences between MS patients with and without cortical lesions in cognitive as well as in neurostructural characteristics other than cortical lesions.

6.2 Topological cortical thinning and executive dysfunction

Studies on executive functions in MS are rare, even though such impairments have been observed in 15-20% of affected patients. Furthermore, their neural correlates remain largely unexplored in MS. Despite the huge amount of correlation studies between different structural alterations and broadly defined cognitive dysfunction in MS (for a review see Rocca et al. (2015) or Chiaravalloti and DeLuca (2008)), focal-topological studies on specific cognitive functions are missing. Based on this research gap, the question about the global and focal neurostructural underpinnings of executive dysfunction rose (second research question; see Chapter 3). With the second study we tried to answer this question.
In summary, the results of the second study revealed two important findings: First, on the behavioral level, we found that executive dysfunction in MS patients appears to specifically affect fluency. It is known that a range of executive functions is affected in MS patients and that some aspects of executive functions can be more severely affected than others in the same individual. Our findings confirm assumptions from Henry and Beatty (2006) who stated that tests of verbal fluency are more sensitive to impairment in MS, relative to other measures of executive functioning.
Second, we showed a lateralized clinico-anatomical correlation pattern between fluency performance and cortical thickness in the anterior cingulate cortex, a finding previously described in brain-damaged patients with large lesions only, for example after stroke. The lateralized pattern described here appears novel and quite unique in the literature on MS. This is in particular interesting, as MS is a disease characterized by a widespread and largely symmetric pathology on both behavioral and neuroanatomical level.

6.3 Randomization performance in patients with MS and its neural correlates

Random number generation (RNG) has been recommended as a potentially powerful tool for monitoring healthy subjects' frontal executive functioning and their attentional capacity. The behavioral pattern found in recent studies are strikingly similar, showing subject-generated sequences to be far from ideal/mathematical randomness. Our third research question (Chapter 3) emerged from the fact that, despite an increasing number of behavioral and functional neuroimaging studies on RNG in healthy subjects and a number of patient group, no study ever investigated

the performance of MS patients in a RNG task. The neuroanatomical correlates of RNG performance have also remained entirely unexplored. Consequentially, the aim of our third study was two-folded. First, the study set out to examine the impact of MS on randomization performance. Second, we wanted to explore the anatomical correlates of RNG. We predicted an impaired randomization performance by the MS patients relative to healthy controls as well as a considerable correlation between behavioral parameters and structural disease characteristics, such as atrophy and lesion indices.

The findings of the study support these two hypotheses. First, we showed that random number sequences generated by MS patients are more stereotyped than those of healthy control subjects. These results are in line with findings of previous studies, showing that sequences generated by brain damaged patients are less random than these generated by healthy controls. In particular, a pronounced counting tendency is a frequent finding in brain injured patients (e.g. Brugger et al., 1996; Brown et al., 1998). Deviations from 'true' sequential randomness are typically ascribed to the limited capacity of working memory and executive functions (Baddeley et al., 1998; Baddeley, 1966).

Beside the sequential non-randomness, patients exhibited - in contrast to healthy controls - also problems in the non-sequential parameters, namely in keeping the rhythm of paced number production (1Hz) and in not naming numbers laying outside the sampling interval. The former difficulty we consider a problem of speed and thus consider the RNG task a useful tool for a brief and valid assessment of information processing speed. Also, the literature suggests that the pattern of RNG performance in humans is unlikely to be driven purely by a limitation in executive functions. Instead, there is evidence that randomization performance of RNG requires considerable allocation of attention (Jahanshahi et al., 2006). Second, the behavioral-anatomical correlation analyses revealed that increased counting in MS patients is associated with a wider third ventricle and higher cortical lesion load. Moreover, an association between rhythm violations and widespread cortical thinning was observed.

6.4 Memory, executive and speed deficits

For a long time, cognitive impairment in MS has been considered less important than other types of impairment such as motor disability. Until the 1980s, it has been thought of as a variant of subcortical dementia with a characteristic set of deficits (Rao, 1986; Beatty et al., 1989). Currently, it is clear that MS-related cognitive impairment has many facets. The clinical and neuroscientific understanding of MS, and its associated cognitive deficits, has been substantially extended

over the past 25 years. In other words, MS-related cognitive impairment seems much more global than would be implied by the concept of 'subcortical dementia' (Thornton & Raz, 1997). The three domains most commonly affected are processing speed, memory and executive functions (Chiaravalloti & DeLuca, 2008). The results of the three studies included in this dissertation are in line with the 'modern' view of cognitive impairment in MS patients. Thus, the cognitive profiles arising from the three studies cannot be defined as purely subcortical, but neither as purely cortical. Besides, memory, executive and speed deficits figured as the most commonly affected cognitive domains also in the present studies. Thus, not all aspects of cognition are affected to the same extent, making it even harder for a clinician to detect and monitor the cognitive decline in MS patients.

6.5 Relevance of cortical pathology in MS

Partly based on findings revealed by modern neuroimaging techniques, the classical view of MS as an inflammatory demyelinating disease affecting primarily the WM of the CNS was extended. It is now established that MS pathology also includes neurodegeneration, and that GM structures such as the cerebral cortex can also show focal lesions, atrophy, or both. Here we presented three studies, which investigated the neural correlates of different cognitive aspects. In all three studies, cortical pathology - be it atrophy or lesions - was associated with behavioral performance impairment. Considering the conflicting observations on the role played by subcortical WM damage in determining the cognitive decline in MS ('clinico-radiological paradox', Barkhof (2002)), our data further strengthen the notion that WM lesions do not fully account for the severity of cognitive impairment in MS, but rather indicate that cortical pathology as one of the major structural changes associated to MS-related cognitive dysfunction. In conclusion, in the last decade much clinical and MRI research has convincingly demonstrated the importance of cortical damage in MS. Thus, cortical pathology should be considered for diagnostic purposes and should be included among the diagnostic criteria for this devastating disease.

6.6 Strengths and limitations of the present studies

Besides the study-specific strengths and limitations discussed in the respective papers, some general aspects should be mentioned here.
Many studies investigating cognitive impairment in MS are lacking a comprehensive carefully selected neuropsychological set of tests. Most studies covered a

limited range of neuropsychological tests or report only a global cognitive index. Furthermore, data from a control group are often lacking. We used a broad number of standardized and validated tests, assessing a variety of cognitive functions. The single tests were administered to a large cohort of MS patients (N=48) as well as to a control group (N=48) in a standardized manner. The latter was homogeneous and matched in terms of demographical (age, gender) and educational characteristics with the patient group. In contrast to many studies of the current MS research using random cut-off points - determined by the researcher - to define cognitive impairment, we used the matched control group in order to compare our findings. Random cut-off points as well as the variability of assessment tools are leading to a reduced inter-study comparability and reproducibility. Therefore, a general agreement in defining reliable cut-off points for cognitive impairment would be desirable.

A minimal education of an apprenticeship, i.e. 12 years, was an inclusion criteria for all subjects in all studies, which may have resulted in a overrepresentation of well-educated subjects in our cohort. According to the cognitive reserve hypothesis, stating that greater lifetime intellectual enrichment lessens the negative impact of brain disease on cognition (Stern, 2002, 2009), this overrepresentation of well-educated subjects may have biased the cognitive performance of our patient sample. Moreover, seriously affected patients were excluded from the study. In summary, the inclusion of well-educated patients with only a mild to moderate affection might reduce the representativeness of the cohort for MS patients at large.

Regarding the post processing of the MR data, some methodological challenges need to be emphasized. For the measurement of cortical thickness, brain tissues have been automatically segmented by classifying each vertex into WM, GM or CSF. This segmentation is prone to error, because of the variable contrast of tissue borders. Especially a high lesion load can cause problems and influence the segmentation of WM and GM. With the visual inspection of the segmentation of each subject, we were able to detect misclassifications. However, in only two patients a manual correction was need due to grossly erroneous segmentation.

As stated in section 4.3.1, quantitative characterization of MS lesion load is of central importance to clinical studies investigating the impact of lesions on cognitive abilities. Even though fully automated methods are often applied, they are error prone and less accurate than the manual segmentation of MS lesions. Thus, in the studies presented here, lesion quantification was performed by a careful manual delineation of all lesions. However, this method - in contrast to automated lesion marking - is potentially the source of a different set of methodological shortcomings. A variety of experimenter effects, conscious or unconscious to the image rater, could have biased the findings.

6.7 Clinical implication

Given the devastating effect MS-related cognitive impairment can have on working capacity or general quality of life, the need for targeted interventions is evident. The main driving motivation of this doctoral thesis was the need to better explain the complex manifestation of cognitive impairment, as cognitive capacity is critical for a range of daily activities. Cognitive impairment results in considerable disruption of lifestyle, social and vocational activities, employment status and level of independent living by causing problems even in routine household tasks. Improving the knowledge of the pathophysiology of cognitive impairment in MS and the identification of the mechanisms responsible for its evolution over time, will contribute to the development of improved and efficient treatment strategies. Moreover, the identification of target structures helps to develop innovative pharmacological and behavioral therapies to enhance cognitive function in MS, which are still scant. Thus, the need for increased awareness of cognitive impairment is essential for the development of effective therapy concepts and rehabilitation programs. Assuming that cognitive decline emerges early in the disease course, early detection and therapy may prevent from deterioration of cognition and may help with treatment decisions.

6.8 Implications for future work

The present work confirms and extends previous research investigating the neuroanatomical underpinnings of cognitive impairment in MS. While there is an extensive cross-sectional literature pertaining to the prevalence and pattern of cognitive impairment in MS, much less is known about the rate and pattern of cognitive deterioration that occurs over the course of illness. Furthermore, only few parameters have been identified as a predictor for cognitive impairment. Thus, additional markers that could be assessed early in the disease are badly needed. Imaging characteristics may provide crucial information on the evolving disease and the development of cognitive impairment. By aligning longitudinal study designs, future studies including broad neuropsychological and multiparametric MRI assessment are mandatory to provide new and important insights of the pathogenic underpinnings of cognitive impairment in MS.

With today's imaging techniques, it is only possible to visualize the 'tip of the iceberg' of cortical MS lesions (Seewann et al., 2011). Further progress in detection algorithms and the development of new MRI sequences and modern imaging techniques can be expected and will likely improve our understanding of MS pathology, symptoms, and treatment.

Furthermore, only recently MRI research in MS has drawn its attention to subcortical structure changes and its association with cognitive impairment. Although volume loss in the basal ganglia and thalamus has been identified, only little is known about its association to cognitive impairment and its progression. Thus, well-designed correlational studies might identify the role of subcortical changes and the respective structure-function relationship.
Research from the past few years highlights the significance of lesion-independent normal-appearing WM abnormalities, which is characterized by the presence of axonal spheroid and swellings, mild inflammation, microglial activation, gliosis and increased expression of proteolytic enzymes. An association with cognitive dysfunction has been proposed. Abnormalities of the normal-appearing WM and diffuse injury to GM, assessed with quantitative MRI techniques, are important in determining cognitive impairment in patients with MS. Thus, structural connectivity analyses like fiber tracking will be needed to more fully understand MS-related WM fiber abnormalities.
Regarding cognitive dysfunction there is also some work to be done. Since the clinical manifestation of MS-related cognitive impairment is not sufficiently described by the traditional 'single pattern' concept of subcortical dementia, the development of a typology of several neuropsychological MS profiles is worthwhile. Therefore, large datasets that include comprehensive cognitive tests and questionnaire results as well as MRI markers are needed. This approach could be pursued by a structural equation modeling approach building composite scores (Ullman, 2006). It seems reasonable to develop such typology in a widespread and inhomogeneous disease such as MS, instead of describing a single pattern or parameter. Such typology might, for example, facilitate prognosis, risk stratification, and treatment recommendations.

6.9 Conclusion

The clinical and neuroscientific understanding of MS has been substantially extended over the past 25 years. In addition to the notion of an inflammatory demyelinating WM disease, it is now established that the MS pathology includes neurodegeneration, and that the GM of the CNS can also be affected. Embedded in this paradigm shift, neuropsychological studies have revealed that cognitive impairment is frequently observed in all MS subtypes at all stages of the disease, and that its clinical manifestations are heterogeneous. With the recognition of the importance of cognitive functioning in MS arises a responsibility on the part of both clinicians and researchers to seek to increase understanding of these cognitive

deficits. With this doctoral thesis we make a significant contribution to the research field of cognitive impairment in MS patients by adding a small puzzle piece to the immense work already done.

In line with the paradigm shift, the relevance of cortical pathology for cognitive impairment has been highlighted in the present work. Moreover, it has been elaborated on different aspects of cognitive impairment in MS patients and its underlying neural correlates: 1) The appearance of cortical lesion in mesiotemporal structures is associated with memory deficits, 2) cortical thinning in the anterior cingulate cortex predicts fluency deficits and 3) widespread cortical thinning and cortical lesion load - together with central brain atrophy - correlate with poor performance on a random number generation task, engaging a number of executive functions and processing speed.

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Publications

Geisseler, O., Pflugshaupt, T., Bezzola, L., K. Reuter, Schuknecht, B., Weller, D., Brugger, P., Linnebank, M. (2016). Cortical thinning in the anterior cingulate cortex predicts multiple sclerosis patients' fluency performance in a lateralised manner. NeuroImage: Clinical, 10, 89-95.

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Research Grant

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